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CHROMOSOMES IN OSMUNDA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 132

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(WITH PLATE 1)

This paper presents the results of a study of the behavior of chromosomes during both homotypic and heterotypic mitoses in *Osmunda cinnamomea* L.

The material was collected in the vicinity of Chicago, Illinois, in the late summer of 1906 and the spring of 1908. The study was made on both living and fixed material. Fixation was most satisfactory in Flemming's weak solution containing osmic acid. The chromosome conditions were studied not only in vegetative mitosis in sporophytes, and in sporogenesis, but also in germinating spores and in mature prothallia. This paper, however, will be limited to a brief account of chromosomes in the sporophyte.

Papers dealing with the cytology of Osmunda have been published by HUMPHREY (**7**), STRASBURGER (**15**), SMITH (**14**), FARMER and MOORE (**2**), and by GRÉGOIRE (**4**). These authors have all chosen *Osmunda regalis*. HUMPHREY and SMITH devoted their attention chiefly to the achromatic substance; STRASBURGER studied sporogenesis and the number of chromosomes; FARMER and MOORE claimed that the bivalent chromosomes arise by a folding of the spirem; and GRÉGOIRE studied chiefly the structure of the double spirem of synapsis, which he found to originate by the association of two independent chromatin threads. GRÉGOIRE'S account is in accord with the present study of *Osmunda cinnamomea*.

The cytological investigation was carried on in the Hull Botanical Laboratory of the University of Chicago, under the direction of

Professors JOHN M. COULTER and CHARLES J. CHAMBERLAIN, and I wish to express my sincere gratitude for their kind suggestions and criticism during the progress of the work.

Description

CHROMOSOMES IN TELOPHASE OF VEGETATIVE MITOSIS

In order to make a detailed study of the behavior of the chromatin material throughout nuclear division, it is necessary to begin with the study at the earliest possible stage of division. To begin with the resting nucleus is not early enough, and so I have started at the telophase of the previous division.

The nucleus in the vegetative tissue, whatever its location may be, presents similar features, so that the description can be applied to the process of nuclear division in any tissue, although the figures in the accompanying plate were drawn from vegetative mitosis in young fronds, previous to the formation of spore mother cells.

The chromosomes in the equatorial plate in typical mitosis generally appear homogeneous. They split longitudinally and the two sets of daughter chromosomes begin to pass toward the poles. The slender and straight daughter chromosomes always retain this form until they reach the poles, where they are drawn more closely together and become more or less parallel. They remain for a while aggregated thus, in contact with the surrounding cytoplasm. If the chromosomes in this state could be called a nuclear primordium, evidently the daughter nucleus in the telophase consists of chromatin only. Then the process of vacuolation begins as follows.

The loosely aggregated chromosomes draw near together and come into closer contact; at the same time each gradually loses its hitherto compact structure and vacuolation occurs irregularly at different places (*fig. 1*). The set of daughter chromosomes is thus a mere aggregate of vacuolate chromosomes. The limits of the individual chromosomes are not difficult to trace. The vacuolation seems to mean that there is either a secretion of fluid from the chromosomes or a dissolution of portions of them into liquid; and the contact of this fluid with the surrounding cytoplasm may precipitate a membrane which will separate the products of the vacuolate chromatin from the cytoplasm. The daughter nucleus formed in this

manner has no possible chance of including achromatic substances, so that the only substances within the nuclear membrane are the chromatin and substances derived from chromatin. This is in accord with GRÉGOIRE and WYGAERTS' results (**5**).

At the beginning of the process of vacuolation the chromosomes do not lie strictly parallel, but converge toward the pole (*fig. 1*). Naturally, chromosomes thus aggregated leave unoccupied space at both their convergent and divergent ends; and during the process of vacuolation a nucleolus always appears in the young nucleus at or near the vacant space in the nuclear cavity beyond the convergent ends of the chromosomes (*fig. 2*). Such a manifestation of polarity is usual in the telophase of typical mitosis.

By careful observation the limits of each of these chromosomes are discernible for some time. When vacuolation accompanied by nuclear growth has proceeded still farther, and the chromatin networks resulting from the individual chromosomes have become connected one with another, so as to appear like a single network irregularly distributed throughout the nuclear cavity, the polarity is no longer recognizable (*fig. 3*), and this is regarded as the resting state of the nucleus.

The nucleus in the resting state is a reticulum, consisting of ragged clumps and strands of irregular shape. The clumps and strands are chromatin; the former are more deeply colored by stains than the latter, not because they are substances of a different nature, but simply because of differences in density.

The number of the chromatin clumps in the resting nucleus in Osmunda is large and variable, always far greater than the number of chromosomes. Without doubt, certain areas of these clumps and strands may represent the limits of certain chromosomes in the resting condition, but even after tracing a very close series of stages from the early telophase to the resting nucleus, the limits of individual chromosomes in the resting reticulum are difficult or impossible to discern.

FORMATION OF CHROMOSOMES IN VEGETATIVE MITOSIS

In early prophase the chromatin of the resting state, composed of fine ragged clumps and strands, becomes more and more evident

at certain parts, possibly by translocation of material from other parts. This gradual translocation of material tends to produce, out of the reticulum, smoother and smoother threads of somewhat uniform thickness, extending for some distance without branching.

Such smooth strands are formed here and there from different parts of the reticulum. Of course, for some time these strands bear fine fibrils by which the various strands are connected into a single nuclear network (*fig. 4*). But finally these fine fibrils, which consist of chromatin, become disconnected; evidently the material is drawn into the strands, which naturally grow thicker on this account. The strands represent an early stage of the somatic chromosomes. When the chromosomes are just organized as a number of independent elements (*fig. 5*), they are slender and very much curved, evidently lying in the position where they had first arisen out of the network as smooth thick strands.

Owing chiefly to the curved nature of the chromosomes at their first appearance, it is difficult to prove that the place where a chromosome first appears is identical with the limit of the chromosome when last distinguishable in the telophase of the previous mitosis. This does not prove, however, that a chromosome may not appear in prophase in the same position in which it was last seen in the preceding telophase.

LONGITUDINAL SPLITTING OF SOMATIC CHROMOSOMES

The chromosomes thus formed are homogeneous and are strictly univalent during the prophase. The longitudinal splitting is first indicated very late in the prophase, just before arrangement at the equatorial plate. The process of longitudinal splitting is gradual and slow. Each chromosome, which has been compact throughout, becomes rather faintly stained in the central region, where the density of the aggregated chromosomes becomes less, although no change has taken place in the contour (*fig. 6*). Then contractions occur simultaneously along the two lateral lines on opposite sides of the strand, where the structure has already become looser (*fig. 7*). The constriction proceeds inward from the two opposite lateral lines and meets in the center, thus dividing the chromosomes longitudinally into two similar halves. The longitudinal halves of each of these

chromosomes, separating at the equatorial plate, proceed to the poles and the vacuolation process follows, as already described.

FORMATION OF CHROMOSOMES IN HETEROTYPIC MITOSIS

The origin of the chromosomes in the spore mother cell is entirely different from that in vegetative mitosis. Some facts are well known and there is an immense literature based upon various material, but the extensive literature does not necessarily mean that all questions have been settled; on the contrary, opinions and interpretations are still conflicting.

Although the nucleus of a spore mother cell in the resting state does not appear very different from that of a vegetative cell, it has characteristic differences, such as its immense increase during the growth period, and the behavior of the chromatic substances outside the nucleus. The most important difference, however, is seen inside the nucleus, in connection with the origin of chromosomes.

The chromatin network in the resting state, consisting of irregular ragged clumps and strands, at first begins to be transformed into more or less regular and less ragged strands, which are uniform in thickness for some distance. These strands are developed simultaneously in various parts of the chromatin network, and at the very beginning of the transformation each chromatin thread thus formed has a thread running parallel to it; in other words, the threads come out of the network as two independent threads from the start.

The pairs of threads at their first appearance are connected by fine fibrils, by means of which all these threads are connected into the single framework of the nucleus. As the delicate connecting fibrils become less and less conspicuous, the duality of the threads is shown with more clearness. The course of the threads being irregularly curved, it is hardly possible to determine their number at this time. The number is certainly less than the reduced number of chromosomes, and there may be only a single pair of threads.

A close examination of the double threads or spirems in this stage shows that they are not uniform in density or in thickness, but the chromatin material is distributed irregularly, so that the parts where it is less densely aggregated stain lighter than the parts where it is denser (*fig. 8*). The knots in one of the double threads do not

necessarily lie side by side with those in the other; in other words, there is no uniformity in the relative position of the knots in the two parallel threads.

The double threads, having such a structure and traversing the nuclear cavity in various directions, now become tangled in a mass at one side of the cavity in the condition called synapsis (*fig. 9*). Synapsis is not an artifact, but a normal stage of prophase, which may be observed in living material. The position of the nucleolus at synapsis is variable; sometimes it lies at a distance from the synaptic mass, but more often it is caught in the tangle. Its form is generally spherical.

Very frequently it occurs that in the tangled mass many parts of the threads are seen converging to that point where the mass is in contact with the nuclear membrane. A similar condition is described by GRÉGOIRE in *O. regalis* (**5**). In this case, some of these parts are continuous with the other parts, and evidently they do not yet represent chromosome primordia already disconnected and independent.

The chromatin structure of the double threads, as seen in the presynaptic stage, is kept throughout this synaptic condition; the two members of the pair may come into closer association in some places than before, but the duality is never lost, even in the culmination of synapsis (*fig. 10*). This means there is no actual fusion of the two threads.

The synaptic mass then begins to disentangle and the double threads again assume a position traversing the entire cavity, the two being always clearly in close association. Each element of the double thread then shortens. During the strepsinema stage some parts of the double threads gather somewhat at a part of the nuclear cavity, looking like a second contraction stage (*fig. 11;* in which only a part of the threads are represented, as they are seen in one focus). Soon after this stage the double threads rapidly shorten and thicken, and finally in a diakinetic stage there are formed 22 bivalent chromosomes (*fig. 12*). In some cases the two elements of each bivalent chromosome remain in close contact, but in other cases they become somewhat separated, and, as a consequence, there are produced the various familiar aspects of bivalent chromosomes.

After the formation of the bivalent chromosomes, the shortening

and thickening proceeds until metaphase, when the structure, instead of being of irregular density, becomes evenly compact and homogeneous. The two chromosomes of the pair separate in metaphase (*fig. 13*) and proceed during anaphase to the poles. But before they reach the poles, there occurs a genuine longitudinal splitting of the univalent chromosomes in preparation for the second division. The splitting generally does not proceed throughout the whole length of the chromosomes, one end remaining unsplit and the parts already divided diverging so that there naturally results a V-shaped chromosome. Therefore in the heterotypic division there is no longitudinal splitting of chromosomes. The two chromosomes lying side by side simply separate at metaphase; there is, of course, a single longitudinal splitting in metaphase of the heterotypic division, but this is a provision for the second division.

The V-shaped chromosomes in late anaphase of the first division (*fig. 14*) gather into a group at the pole. Vacuolation occurs and a nuclear membrane is formed.

FORMATION OF CHROMOSOMES IN HOMOTYPIC MITOSIS

The group of vacuolate chromosomes is distinctly recognizable after the formation of the nuclear membrane. As the vacuolation proceeds farther, the chromosomes become very alveolate, but as the process is more active in the lateral parts of each chromosome, the central part remains as a rather thick strand, so that for a considerable period after the organization of the daughter nucleus the V-shaped chromosomes could be traced with perfect distinctness.

Progressive vacuolation with the accompanying nuclear growth tends to change the general aspect of the ragged chromatin network of the newly formed nucleus. The process, however, before proceeding so far as to result in a resting stage, begins to reorganize the chromosome primordia out of the ragged chromatin reticulum.

The chromosome primordium appears first in V-shape (*fig. 15*), but the location does not seem to agree exactly with the location of the daughter chromosomes as last seen in the previous telophase. In the nuclear cavity, during the period intermediate between the last telophase and the present prophase, there might have occurred some movement of parts of the chromatin network. But the uni-

formity in the number of chromosomes and their appearance as exactly V-shaped as when they entered into the alveolate and reticulate condition, seem to indicate strongly that the limits of the individual chromosomes are distinctly maintained during the nuclear changes.

After the disappearance of the nuclear membrane the divergent arms of the V-shaped chromosomes draw near to one another, and as they are arranged in an equatorial plate the two arms lie closely parallel. In metaphase the two arms separate. The two sets of daughter chromosomes reach the poles and vacuolation begins (*fig. 16*); their aspect in this stage is like the telophase of the vegetative mitosis, except in the number of chromosomes.

Discussion

The object of this paper is to present briefly the results obtained in the study of the chromosomes of *Osmunda cinnamomea*, and no detailed discussion of the literature of the subject is intended. Only a few remarks on the morphology of the chromatin substance will be made at this time.

SYNAPSIS

Although there are a few authors, as McClung (**9**), Schaffner (**13**), Janssen (**8**), and Haecker (**6**), who believe that synapsis is either an artifact or has no significance in the reduction division, yet the phenomenon has been demonstrated in many cases in living material, and now a majority of workers agree that the stage is of perfectly normal occurrence and that it always directly precedes the reduction division.

The details of synapsis in the plant cell have been followed by various botanists in a great number of plants, the most detailed accounts relating to flowering plants. Excepting Strasburger's *Gamosomen* theory based on his study of Galtonia (**16**), many botanical cytologists agree that there are parallel nuclear threads. As regards the structure of the nuclear threads the views differ; some (Farmer and Moore **2**, and others) believe the threads to be composed of chromatin imbedded in linin groundwork; some (Overton **11**, and others) claim that it is composed of prochromosomes connected with the

linin intervals; and still others (GRÉGOIRE **3**, and others) conclude that the threads are composed exclusively of chromatin.

Regarding the origin of the parallel threads, FARMER and MOORE (**2**) and others believe they result from a longitudinal splitting; GRÉGOIRE (**4**), ALLEN (**1**), and others contend that the two are associated but independent; and the interpretations of synapsis naturally differ according to the views held in regard to the structure and origin of the nuclear threads.

In Osmunda, although the chromosomes during the resting period undergo changes so that their form differs from that seen during division, tracing the progressive changes makes it seem probable that the vacuolation does not destroy the individuality of the chromosomes. On the contrary, the individual chromosomes are preserved as vacuolate and alveolate masses during the resting period, and they again reappear in compact form at the next division. The interval between the heterotypic and homotypic divisions is much shorter than the period occupied by their divisions, and the V-shaped chromosomes reappear in the exact V-form in which they entered into the formation of the network.

These somatic chromosomes are of maternal and paternal origin, and they have come to be included within a common nuclear wall at the time of fertilization. After fertilization, during the succeeding mitoses, the individuality of chromosomes is thus maintained, and there is no time when these maternal and paternal chromosomes come into contact as regularly formed chromatin threads until the time of synapsis. How much difference there exists between the association of maternal and paternal chromatin material in the vacuolate and alveolate condition and in the form of regularly ordered chromatin threads cannot be suggested; but the importance of synapsis as occurring only once in the cycle of chromosome history, directly preceding the reduction division, cannot be overestimated.

ORIGIN OF HETEROTYPIC CHROMOSOMES

The heterotypic chromosomes in Osmunda arise as independent pairs at the early prophase of the reduction division. This result is in accord with the views held by GRÉGOIRE (**4**), ALLEN (**1**), ROSENBERG (**12**), OVERTON (**11**), and others. However, the method of

forming heterotypic chromosomes in Fucus (YAMANOUCHI **17**) is different; the threads seemed to indicate no association at the beginning of early prophase, and even after they become tangled in a mass at synapsis parts of them seem to be single. In the post-synaptic stage a reduced number of loops is formed from the threads. Evidently the bivalent chromosomes are formed from the two associated arms of each loop. Unless there be some failure in observation, there must be two ways of forming heterotypic chromosomes. If the association of parental chromosomes occurs in the regularly formed chromatin threads in synapsis, the end-to-end hypothesis, held by FARMER and MOORE (**2**), SCHAFFNER (**13**), MOTTIER (**10**), STRASBURGER (**16**), and others, seems to be the correct interpretation.

Probably there may be more than one series of details in mitosis, and it would be too hasty to make any generalization from comparatively few observations. The present account simply deals with the observations upon *Osmunda cinnamomea*.

Summary

1. The reticulum in the young nucleus arises fron the chromosomes of the previous division by vacuolation. It consists chiefly of chromatin material.

2. The chromatin network during the resting stage shows no indication of a pairing of knots or strands.

3. Individuality of the chromosomes is retained in the vacuolate and reticulate form during the resting stage, although the limits of individual chromosomes become hard to trace.

4. The pairing of chromatin material, perhaps of maternal and paternal derivation, appears only at the early prophase of heterotypic mitosis. The pairs may come into the closest association during synapsis, but the duality is maintained. As a consequence no actual fusion occurs.

5. There is no splitting of chromosomes in the heterotypic mitosis; each bivalent chromosome is formed by the association of two independent chromosomes. The separation of the two gives an appearance of longitudinal division.

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17. YAMANOUCHI, S., Mitosis in Fucus. BOT. GAZETTE **47**:173–197. *pls. 8–11*. 1909.

EXPLANATION OF PLATE I

The figures are drawn with the aid of an Abbé camera lucida, with Zeiss apochromatic obj. 1.5^{mm} N. A. 1.30, combined with compensating ocular 18,

except *figs. 1, 15, 16*, drawn with compensating ocular 12, and *figs. 8, 9, 10, 11, 12*, drawn with compensating ocular 8. The plate is reduced to two-thirds the original size.

Figs. 1–7.—Vegetative mitosis in the young sporogenous tissue

FIG. 1.—Vacuolate chromosomes in late telophase.

FIG. 2.—A young daughter nucleus with the manifestation of polarity by the location of the chromatin network and a nucleolus.

FIG. 3.—A portion of the chromatin reticulum in the resting nucleus.

FIG. 4.—A part of the chromatin threads arising from a ragged chromatin reticulum.

FIG. 5.—A nucleus in which homogeneous chromosomes are just organized.

FIG. 6.—Portions of chromosomes from an equatorial plate; the chromatin material in the central region has become less compact.

FIG. 7.—Portions of chromosomes in a later stage than *fig. 6;* constriction has begun along two lateral lines.

Figs. 8–16.—Mitosis in the spore mother cell

FIG. 8.—Portions of double threads from the nucleus a little before the leptonema stage; chromatin material of different density in different parts of the threads.

FIG. 8*a*.—A spore mother cell with a nucleus whose chromatin is shown under higher magnification in *fig. 8*.

FIG. 9.—Portions of double threads from the nucleus in a climax condition of synapsis (so-called zygonema stage).

FIG. 9*a*.—A spore mother cell with a nucleus whose chromatin threads are shown under higher magnification in *fig. 9*.

FIG. 10.—Portions of chromatin double threads from the nucleus in pachynema stage.

FIG. 10*a*.—A spore mother cell with a nucleus whose chromatin threads are shown under higher magnification in *fig. 10*.

FIG. 11.—Portions of double threads in strepsinema stage; the independent two have begun to separate.

FIG. 11*a*.—A spore mother cell with a nucleus whose chromatin threads are shown under higher magnification in *fig. 11*.

FIG. 12.—Portions of chromosomes in diakinetic stage.

FIG. 12*a*.—A spore mother cell with a nucleus whose chromosomes are shown under higher magnification in *fig. 12*.

FIG. 13.—Portions of chromosomes with spindles in an equatorial plate.

FIG. 14.—Portions of chromosomes with spindles at the anaphase of the heterotypic division; longitudinal splitting in each chromosome.

FIG. 15.—Early prophase of the second division; the V-shaped chromosomes are distinctly recognizable.

FIG. 16.—Telophase in the second division.

YAMANOUCHI on OSMUNDA

THE DEVELOPMENT OF THE EMBRYO OF ENCEPHALARTOS

W. T. SAXTON

(WITH PLATE II AND ONE FIGURE)

Toward the close of 1906 Dr. H. H. W. PEARSON offered to turn over to me some material collected[1] with a view to following the development of the proembryo and embryo of Encephalartos. We hoped to publish a joint paper, of which the present account, completed two years ago, was to have been my contribution. As Dr. PEARSON now finds that the publication of his share will have to be delayed still further, he has suggested that I should publish the account separately. I am glad to take this opportunity of thanking him for his help, and especially for the drawings to which his initials are appended. I also wish to thank Professor SEWARD for assisting me with the microphotograph from which *fig. 18* is reproduced.

All sections were cut with a Cambridge rocking microtome, except that from which *figs. 16* and *17* were drawn, which was cut by hand. All figures were drawn with the aid of a Zeiss camera lucida, microscope, and lenses, except *fig. 18*, which is from a microphotograph. *Figs. 1, 2, 6–8, 12–15* were drawn by Dr. PEARSON, the others by myself.

The material at my disposal afforded a fairly complete series of the development of the embryo, but the stages to illustrate the proembryo and early post-fertilization stages are almost entirely wanting and will not be referred to. The first account of embryo development in cycads seems to have been that by WARMING[2] for Ceratozamia, but only a very few stages are described and figured. Proembryo development has been described in Cycas by TREUB[3] and

[1] With the aid of a grant from the British Association for the Advancement of Science.

[2] WARMING, E., Undersogelser of Betragtninger over Cycadeerne. 1877.

[3] TREUB, M., Recherches sur les Cycadées. 3. Embryogénie du *Cycas circinalis*. Ann. Jard. Bot. Buit. **4**:1–11. *pls. 1–3*. 1884.

IKENO,[4] and in Zamia by COULTER and CHAMBERLAIN,[5] but later stages have not been closely followed for any cycad, and the results now presented fill in some of the gaps still remaining in our knowledge of this interesting group.

Description

The intraseminal development of the embryo for descriptive pūrposes may be divided into four stages, each of which passes gradually into the next: (1) the differentiation of a small mass of meristematic tissue at the base of the proembryo; (2) the rapid division and elongation of a group of cells at the proximal end of this meristematic tissue, forming the suspensor; (3) the differentiation of the cotyledons and stem apex; (4) the development of plumular leaves and the fusion of the distal halves of the cotyledons.

The youngest stages figured are from the embryos of *Encephalartos villosus* (*figs. 1, 2*). *Fig. 1* represents a stage in the development of the suspensor, the embryo being shaded. *Fig. 2* is drawn from a slightly older embryo, showing the latter in detail and a small part of the suspensor. The small group of actively dividing cells, about 40–50 in number (12–16 in sections), which is forming the suspensor, can be recognized readily by the fact that the cells are arranged in regular longitudinal rows.

The remaining figures (*3–18*) are from embryo of *E. Friderici-Guilielmi*.[6] *Fig. 3* represents the only case met with in which the suspensor was branched, the branches bearing two equally developed embryos. The figure shows the two branches of the suspensor only up to the point of junction; the suspensor was injured in sectioning just above this point, and it is impossible to say whether the branching is dichotomous or monopodial.

Fig. 4 is an outline of a longitudinal section of an embryo at the close of the second stage of its development. The part inclosed by a

[4] IKENO, S., Untersuchungen über die Entwickelung der Geschlechtsorgane und der Vorgang der Befruchtung bei *Cycas revoluta*. Jahrb. Wiss. Bot. **32**:557–602. *pls. 8–10*. 1898.

[5] COULTER, J. M., AND CHAMBERLAIN, C. J., The embryogeny of Zamia. BOT. GAZETTE **35**:184–194. *pls. 6–8*. 1903.

[6] This appears in *Index Kewensis* as a synonym of *E. cycadifolius*, but Dr. PEARSON informs me that he finds the two species to be quite distinct.

dotted line is drawn in detail in *fig. 5*. The group of suspensor-forming cells has now become the root meristem, and is not sharply differentiated from the distal part of the suspensor. At this stage cell division has recommenced in the distal half of the embryo, though as yet no further differentiation has taken place.

The first appearance of the cotyledons is shown in *fig. 6*. They clearly arise, as described for Ginkgo by LYON,[7] by the more rapid growth of two groups of cells of the distal meristem. The cotyledons of Pinus also are initiated by the more rapid growth of groups of cells of the apical meristem,[8] and it is probable that this is normally the case in all gymnosperms. The dermatogen is distinct at this stage, and the root meristem is more clearly shown than in *fig. 5*. The latter point is shown better in *fig. 7*, where the central part of *fig. 6* is drawn in detail.

Figs. 8, *9*, and *10* show later stages, in which the principal changes are the elongation of the cotyledons and the development of the canals. The first indications of the formation of canals can be seen in the stage outlined in *fig. 9*, and their position is indicated in *fig. 10*. The canal marked *x* in *fig. 10* is drawn in detail in *fig. 11*. The dense contents contain tannin, and probably mucilage also, though it is not easy to demonstrate its presence. The canals are formed lysigenously, that is, by the breaking-down of rows of cells. The boundary of the root meristem is indicated by a dotted line in *figs. 9* and *10*. In the stage represented by *fig. 10* the tissue lying between the apical meristem and the root meristem is beginning to lose its meristematic character; the cells of this region are somewhat flattened longitudinally. *Figs. 12–15* show the outline of transverse sections of an embryo of approximately the same age as that drawn in longitudinal section in *fig. 10*. *Fig. 12* shows the base of the cotyledons and their attachment to the stem; *fig. 13* is taken just below the stem apex; *fig. 14* is somewhat higher up; and *fig. 15* is near the apex of the cotyledons. The principal point shown by this series is that at this stage the cotyledons are fused only in the proximal half of their length.

[7] LYON, H. L., The embryogeny of Ginkgo. Minn. Bot. Studies **3**:275–290. *pls. 29–43*. 1904.

[8] The writer hopes to publish later an account of the development of the embryo in Pinus.

FIG. 18.—*E. Friderici-Guilielmi:* microphotograph of part of a longitudinal section of a nearly mature embryo. ×50.

In the last stage of intraseminal development the primordia of one or two plumular leaves reach a moderate size. The cotyledons in the mature embryo are fused throughout their whole length, by both margins at the base, and by either one or both margins near the apex. *Fig. 16* represents a transverse section in which they were fused by one margin only; the part of the section marked *x* is drawn in detail in *fig. 17*. This shows the very intimate union of the epidermal cells and their collenchymatous thickenings.

Fig. 18 represents the root and stem apices, the bases of the cotyledons, and the first plumular leaf. The root meristem has now increased considerably in size, but has not otherwise changed its character in any way, so that what is physiologically the root cap is only the distal end of the suspensor as morphologically equivalent to part of the root cap of any ordinary angiospermous embryo. The embryonal tubes in certain conifers are evidently equivalent to the whole suspensor of Encephalartos.

From the preceding account it is clear that there is a very close resemblance between the embryogeny of Encephalartos and that of Ginkgo as described by LYON (*l. c.*).

Summary

1. The suspensor is developed by the division and elongation of a group of cells at the proximal end of the embryo.

2. This group of cells forms later the root meristem.

3. Branching of the suspensor has been observed once, resulting in the formation of two approximately equal embryos.

4. The cotyledons are initiated by the more rapid growth of two groups of cells of the apical meristem.

5. The canals appear before the differentiation of plumular leaves, and probably contain both tannin and mucilage, being formed lysigenously.

6. A very intimate connection is established between the epidermal cells of fusing cotyledons.

7. The suspensor is morphologically a root cap.

8. The embryogeny of Encephalartos is very similar to that of Gingko.

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CAPE TOWN

EXPLANATION OF PLATE II

Encephalartos villosus

FIG. 1.—Suspensor carrying down young embryo (shaded) into the endosperm; *aw*, archegonium wall. ×77.

FIG. 2.—Young embryo slightly older. ×310.

Encephalartos Friderici-Guilielmi

FIG. 3.—Branching of the suspensor forming two approximately equal embryos. ×77.

FIG. 4.—Outline of embryo and part of suspensor, just before differentiation of cotyledons. ×40.

FIG. 5.—Detailed drawing of part of *fig. 4* indicated by dotted line. ×300.

FIG. 6.—Cell outline of embryo just after differentiation of cotyledons. ×117.

FIG. 7.—Detailed drawing of part of *fig. 6;* junction of root and stem meristems. ×300.

FIG. 8.—Outline of slightly older embryo. ×24.

FIG. 9.—Older embryo than *fig. 8;* root meristem outlined with dotted line. ×40.

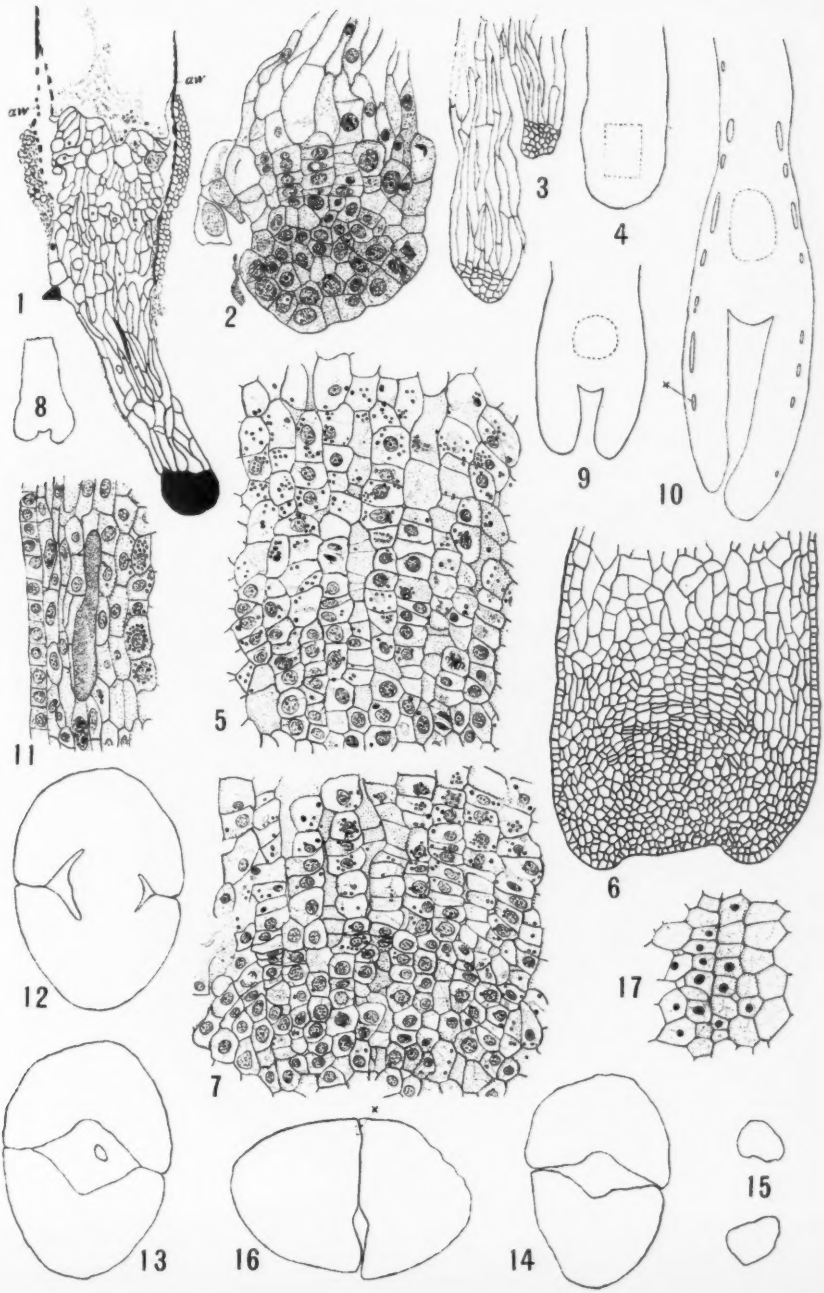
FIG. 10.—Embryo older than *fig. 9;* canals outlined, that marked *x* shown in next figure. ×40.

FIG. 11.—Canal from *fig. 10* drawn in detail. ×310.

FIGS. 12–15.—For explanation see text.

FIG. 16.—Cross-section of cotyledons of a mature embryo. ×24.

FIG. 17.—Part of *fig. 12* marked *x* drawn in detail. ×310.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THE ORIGIN OF HETEROSPORY IN MARSILIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 133

CHARLES H. SHATTUCK

(WITH PLATES III–VI AND ONE FIGURE)

Much morphological work has been done among the heterosporous pteridophytes, but they have not been subjected to experimental methods to obtain some suggestion as to the origin of heterospory. From certain phenomena that I observed in making a morphological investigation of *Marsilia quadrifolia*, it seemed possible that an experimental study might furnish some evidence as to the way in which the heterosporous habit began in this form.

Historical

The natural presumption has been that the Marsiliaceae have sprung from a homosporous ancestry, but there is little definite evidence to substantiate this view. CAMPBELL (**4**) has suggested that a near relationship exists between Marsiliaceae and Schizaeaceae. BOWER (**2**) calls attention to the striking morphological resemblance between the sporocarp of the Marsiliaceae and the spike of Ophioglossaceae.

JOHNSON (**14**), GOEBEL (**11**), and others have pointed out features suggesting that Marsiliaceae are not far removed from the homosporous ferns, but apparently no experimental work has been done which might show that the Marsiliaceae were originally homosporous and by what process they have attained their present heterosporous condition, or in fact how this condition has arisen in any other form.

In 1887 BUCHTIEN (**1**) called attention to the fact that in Equisetum the sex of the prothallium is controlled very largely by nutrition, and drew the conclusion that dioecious prothallia are an indication of incipient heterospory. This view will be discussed later.

The first literature bearing directly upon the origin of heterospory appeared in 1894. WILLIAMSON and SCOTT (**24, 28**), in their work on Calamaria, were the first investigators to offer a tenable theory. After an extensive study of two species of Calamostachys, in which

they found abortion in all of the sporangia in *C. Binneyana* and in only one-fourth of the sporangia in *C. Casheana*, they suggested that the abortion of certain of the spores and the consequent increased nutrition of their surviving fellows may have been the physiological condition that ultimately rendered possible the development of megaspores.

In 1908 THODAY (**27**) added further and convincing evidence from *Sphenophyllum Dawsonii*, to that already furnished by WILLIAMSON and SCOTT (**28**). He shows that the size of the spore varies with the amount of abortion, and that while the average diameter of the spores in this form is 83 μ, the maximum diameter where abortion is extensive is as much as 120 μ, or nearly one-third larger. These are both striking examples of heterospory as it must have appeared in its incipiency, and both show a very definite relation between the size of the spore and the extent of abortion.

Statement

Many have insisted that the only clue furnished as to the possible origin of heterospory is that found in Equisetum, where the prothallia are usually dioecious. This dioecism seems to be due rather to external than to internal conditions, as shown by HOFMEISTER (**13**) as early as 1855, when he pointed out that while the prothallia were for the most part dioecious, they could be made to produce either male or female sex organs, or both, by varying the external conditions. He states that archegonia may appear on late shoots of the so-called male prothallia; while SADEBECK (**23**) shows that antheridia may appear at a later period on the lobes of the female prothallia.

GOEBEL (**12**) says that "it is probable that in this, as in other cases, the male prothallia are those which have been insufficiently fed." BOWER (**2**) calls attention to the fact that the spores of Equisetum show no differentiation in size, or apparently in sex. CAMPBELL (**3**) also states (p. 453) that "external conditions influence the production of males or females as in the ferns, and that while the prothallia are normally dioecious, this is not exclusively the case." I have examined critically several laboratory cultures of the prothallia of Equisetum in which only antheridia were produced in the crowded portions of the sowing. In fact I have grown a number of cultures

in which I could not find a single plant bearing archegonia, though plants bearing antheridia were very numerous. This shows that Equisetum is only slightly more advanced in its tendency to produce dioecious prothallia than the ferns, and that this tendency is largely, if not wholly, controlled by external conditions after the spore begins to germinate, and probably not until the prothallium consists of many cells. This has also been mentioned by PRANTL (**21**) and others. Varied external conditions operating on the plant during the formation and maturation of the spore seem to make no difference in its size or tendency after germination to produce male or female prothallia. In other words, the plant makes no preparation in its spore for the production of female prothallia, the sex being determined long after germination and wholly by chance external conditions.

From a consideration of these facts it appears that the genus Equisetum, as we now know it, furnishes no clue to the origin of heterospory, because it makes no preparation which we can discover in the organization of its spores, either in size, shape, or extra storage of food material for the production of specially large gametophytes. In other words, the sex of the prothallium seems to be determined after germination and wholly by external conditions. In this particular we find it no farther advanced than the true ferns.

When we compare this form with one of the heterosporous pteridophytes, for example Marsilia, we find that the latter plant begins to prepare a few special spores as soon as the tetrad divisions have occurred, which are to produce the female prothallia. These few large spores are produced at the expense of many aborting ones whose substance is gradually absorbed by them. Moreover, these large spores are formed, under normal conditions, in the oldest and most favorably placed sporangia (*figs. 1, 2*), thus showing that here the sporophyte makes definite preparation for the production of spores which are to produce female prothallia.

In the microsporangium every one of the 16 mother cells (*fig. 3*) produces four functioning microspores, 64 in all; while in the megasporangium only one spore matures out of 64, all of which seem to be identical in every particular when the tetrads are first formed (*fig. 4*). A closer examination reveals the fact that this one megaspore does not gain the ascendency without a struggle. In fact, many instances

were found in which there was sharp rivalry between two or more enlarging potential megaspores (*figs. 5–9*). Sometimes this occurs in the same tetrad, occasionally all four enlarging (*figs. 5–7*); sometimes two members of the same tetrad enlarge (*figs. 5, 8, 9*); and sometimes one each of two or more tetrads enlarges. Yet in the end, one centrally placed spore always gains the ascendency, the others becoming abortive.

This apparent plasticity of Marsilia led me to take up a further study of it grown in the greenhouse, and under such conditions that a very careful record of results could be kept. My object was to see what effect varying conditions of light, heat, moisture, etc., might have on the production of the two kinds of spores.

Even among seed plants the development of the megaspore is by no means so uniform as is that of the microspore, even the formation of the well-known tetrad being dispensed with in many forms, such as Lilium, where the mother cell forms four nuclei but fails to develop walls. These nuclei are thought by many to represent four megaspores. We find also that from a single megaspore there may come a varying number of cells within the embryo sac, as found by CAMPBELL (**5**) and JOHNSON (**15**) in Peperomia, where 16 nuclei are formed; and by the writer in *Ulmus americana* (**25**), where 8 to 16 nuclei are formed; and while 8 is the usual number for angiosperms, some plants fail to form even so many, as was shown by Miss PACE in Cypripedium (**19**), where only four are formed.

Also, where the row of four megaspores is formed, there is often a sharp struggle for the mastery; sometimes the lower one, sometimes the upper one, and even one of the middle ones finally functioning as the embryo sac. This is well shown in such forms as Diospyros, in which Miss HAGUE, in an unpublished paper, finds the above conditions. Occasionally two megaspores function, forming two embryo sacs, as shown by ERNST (**10**), and by the writer in Ulmus (**25**) and Pinus (unpublished).

The nuclei within the sac also contend as to which will function as the egg, as shown by CHAMBERLAIN (**6**) in Aster, and by Miss OPPERMAN (**18**), and by the writer (**25**). In contrast with the above varying conditions of the megaspore we find a very constant method of development for the microspore, the mother cell nearly always

forming four spores (rarely 3 or 5), and a very large percentage of these developing, which are very constant in size, number, and behavior.

After reviewing these facts we must conclude that the megaspore condition is a derived condition, that is, derived from the original homosporous condition of the ferns; that it is therefore more recent, and consequently more plastic, and more likely to yield interesting variations when subjected to experimental methods.[1] With these general facts in mind the experimental work was begun.

Methods

In attacking this problem it was first necessary to determine definitely at what stage in its development the contents of the sporangium give positive evidence as to whether a megaspore or microspores are to be formed. JOHNSON (**15**) determined that the megasporangium is the first sporogenous tissue to be differentiated, but did not trace the development much farther. This has been done, and we find that all sporangia have identically the same development until the tetrads are formed (*fig. 4*), at which stage a very slight difference is observed.

In the older sporangia (*fig. 2*), which are also the most centrally located, the four young spores of each tetrad show a marked tendency to hang together by strong protoplasmic strands, as figured by STRASBURGER (**26**) and shown in *figs. 5–10*. These strands are the first recognizable morphological feature by which one can determine that a sporangium is to form a megaspore rather than microspores. They persist until the megaspore is quite mature, and in very many instances can be seen on the papilla of the germinating megaspore, which still subtends the three abortive members of the tetrad (*fig. 10, a*). This condition was figured by WILLIAMSON and SCOTT (**28**) for *C. Binneyana*, although they did not attempt an explanation. In the case of sporangia which were to form microspores it was observed that the protoplasmic strands are not so strong, and that the young spores, while held together during their early development, are in the end separated completely from each other. The fact that the megaspores are held together by stronger protoplasmic strands was first noticed by SACHS in 1866 in *Pilularia globulifera* (**22**).

[1] After this work was well under way Miss PFEIFFER (**20**) reported cases in Azolla where two megaspores have matured instead of one, which is the normal number.

The cause for the varying behavior of the strands in the two kinds of spores was not determined, but the establishment of this fact suggested that any experiments on the plant which might affect nutrition and growth ought to have a more marked influence in determining the kind of spore formed if applied when the sporocarps contained sporangia about ready to form tetrads. The various methods herein described were all found to give the best results if applied at this time.

Various cultures were grown in ponds, in the open air, and in the greenhouse. Careful records were kept covering such points as rate of vegetative growth, color, and vigor of plants, length of petioles, length of time before the appearance of sporocarps, the nature of the sporocarp, the causes producing the largest and most abundant sporocarps, and the causes of blasting of sporocarps and of their complete suppression (details of these cultures will be published in a later paper).

The experimental work was begun in Chicago, June 20, 1905, and continued until the latter part of September; it was repeated again during the same period in 1906; and then carried on continuously from June 1907 until the present time.

On June 20, 1905, I found *Marsilia quadrifolia* growing in Hull Court pond at all depths from 30^{cm} beneath the surface to $20–25^{cm}$ above it on the banks. It was in a most luxuriant condition, some of the rhizomes being more than 2^{m} in length. There was also one tank of growing plants in the greenhouse. Sods from the pond were taken up and transferred to the greenhouse, where they were placed in four large tanks (60 by 90^{cm} and 30^{cm} deep). These were supplied with soil (a rich black loam) spread evenly over the bottom to a depth of 10^{cm}, in which the sods were transplanted at one end of the tank and covered with water to the depth of 5^{cm}. In a few days these tanks were elevated 10^{cm} at one end. This left the soil at the upper end out of water, but submerged the plants, which were at the other end, about 10^{cm}. The tanks were then numbered 2, 3, 4, and 5, and placed under as great a variation of light as was possible to obtain in the greenhouse. All other conditions were kept as nearly uniform in all the tanks as possible, but on clear days there was considerable variation in temperature, as shown by the table of temperature readings.

TABLE I

LIGHT READINGS TAKEN IN OPEN AIR AND IN WATER AT DEPTHS OF 2.5, 5, AND 10cm, AND ALSO IN NORTH AND SOUTH ENDS OF GREENHOUSE

These readings were taken with a solio-paper actinometer and show approximately the varying intensities of the light where different cultures were grown.

				Under water			Greenhouse	
1907	Time	Weather	Open air	2.5cm	5cm	10cm	South end	North end
September 14	3:45	clear	15 sec.	..	..	..	35	65
September 20	12:20–12:40	clear	10 sec.	14	20	..	..	...
September 25	2:00– 2:20	clear (?)	20 sec.	..	..	35	70	210 (shade)
September 26	2:30– 2:40	cloudy	45 sec.	..	..	70	120	170

The plants in the best lighted positions recovered from the effect of transplanting more rapidly and grew much more vigorously than those in more subdued light. This was shown by the rate of growth of rhizomes, which under the most favorable conditions was as much as 12.5cm daily; while for those in the most subdued light 6cm was the maximum daily growth. The color of the plants in good light was also a darker green and the thickness of the leaf blades, rhizomes, and petioles was also greater. Neither the length of the petioles nor the size of the leaves was so great as those of the shaded plants.

On August 6, the first sporocarps were discovered in tank 5, which had been placed in strong light; these were evidently several days old and must have been evident as early as August 3. On August 8, tank 4 showed some sporocarps that had probably been in evidence about two days; while in tank 3 the very first indication of sporocarps was noticed. Tank 2 was in diffused light except for about two hours in the middle of the day, and while the plants grew well they stubbornly refused to fruit either above or below the surface of the water. Finally on August 17 some sporocarps were found in this tank out of water. These soon turned brown, however, and on sectioning were found to be blasted. This tank continued to produce a few blasting sporocarps for 18 days, but it produced good sporocarps as soon as it was placed in a strong light.

Influence of water

It has been observed that *Marsilia quadrifolia* fruits most profusely at the surface or a few centimeters above the surface of the water.

It fruits sparingly under water, and at a depth of 10^{cm} very few sporocarps appear. I observed that most of these blast when about half grown.

In my greenhouse cultures in 1905 I found that all the first sporocarps that appeared in tank 1 blasted when about half grown, except two that happened to be about 8^{cm} higher than the others, due to the uneven surface of the soil in the tank (*fig. 12, m*). After the rhizome passed over this elevation and again sank into 8–10^{cm} of water, the sporocarps began to blast as before. In order to be sure that this was not accidental, on July 5 I lowered the water in this tank until it was not more than 1^{cm} in depth; and then nearly all the sporocarps matured. On July 11 I raised the water to the original level, and by July 16 many of the oldest of the newly formed sporocarps were blasting. On July 21 I again lowered the water to 1^{cm} in depth, and by July 25 the oldest of the sporocarps, formed after that date, had safely passed the period where blasting had occurred. I then put on 20^{cm} of water and found that the blasting occurred after the sporocarp had been visible only about two days, instead of four to six days as observed at the 10^{cm} level. I imbedded and sectioned many of these sporocarps in order to determine the relative vitality of megaspores and microspores. It was from a study of these that I conceived the idea of blasting the megaspores and continuing the development of the sporocarps containing only the surviving microspores. I shall speak later of the methods employed.

I was at a loss to know just what had caused the blasting. While mere depth of water seemed to be the immediate cause, several factors were involved, such as oxygen supply, intensity of light, and temperature, all of which were varied with the change in depth. On August 5 I moved this tank, which had been in subdued light, to the east side of the greenhouse into strong light, and threw a mist of tap water over the plants in order to keep them saturated, but at the same time aerated. This water was siphoned off constantly, so that most of the sporocarps were at the surface. In a few days (August 11) I noted that blasting was occurring quite as universally as before. While the plants now had more light and a better oxygen supply than before, I had lowered the temperature during the middle of the day about 8° and at night about 3°. It was plain that this variation in the

temperature was a very important factor. I then grew the plants out of water for a week without applying the spray, and had no trouble with blasting. This process was repeated several times with the same

TABLE II

OPEN AIR TEMPERATURE READINGS TAKEN FROM 2:00 P. M. TO 3:00 P. M.

1907	Weather	Open air	Pond surface	15cm under water	Tank	Dry soil	Wet soil
August 21.....	cloudy	....	20	23.5	....	....	....
August 22.....	clear	....	23	....	25	....	....
August 23.....	clear	21	20	20	21.5	....	....
August 24.....	clear	..	25	21.5	27	....	....
August 25.....	clear	24.5	27	20	28	35.5	31.5
August 26.....	cloudy	24	22.75	19	24	23	24
August 27.....	cloudy	22.5	23	20.5	24	24	23.5
August 28.....	clear	24	26	22	27.25	29	28
August 29.....	cloudy	20	21	19.25	21	..	22
August 31.....	cloudy	24	28	23	28.5	30.5	....
September 1.....	clear	33.5	30.75	25.5	33.5	33	....
September 14....	clear	....	26	21	27	....	....
Average readings	.....	24.18	24.37	21.39	26.06	29.16	25.8

TABLE III

GREENHOUSE TEMPERATURE READINGS TAKEN FROM 2:00 P. M. TO 3:00 P. M.*

			SOUTH TANK		NORTH TANK			
1907	Weather	Air	Wet soil	Surface	Surface	Tap	Jet	Spray
August 19.....	clear	34	....	35.5	30	15.5	....	....
August 20.....	cloudy	28.5	....	....	20.5	16	....	....
August 21.....	cloudy	25	....	....	....	....	....	....
August 22.....	clear	30	....	....	....	19	30	25
August 23.....	.....	26	....	24	23	....	....	....
August 24.....	clear	30	32	32	23.5	24	34	24.5
August 25.....	clear	27.75	29.75	29.5	....	21	25.5	21.5
August 26.....	cloudy	29	27	27.25	23.5	19.5	31	25
August 27.....	cloudy	25	....	24.5	22	18.75	24	23
August 28.....	clear	30	30.5	28	26	19.5	31	23.5
August 29.....	cloudy	22	22	21.5	....	20	22	20.5
August 31.....	cloudy	33	33	30	28.5	19	35.5	28
September 1.....	clear	39.5	35.25	34.5	33	....	41	26
September 14.....	clear	32.75	....	30	27	....	34	28
Average readings		29.45	29.93	28.8	25.7	19.22	30.8	24.5

*It will be noted that this table of readings was taken in 1907. It is more complete than the one taken in 1905, but does not vary materially from it, except in the columns marked jet and spray. These are higher in this table because the water was heated by means of the sun which shone on about 12m of the pipe, raising the average reading for the jet 9°, and for the spray 4°. The spray was in the north end of greenhouse in 1905, close to the riser pipe; and the temperature of the jet was very little above the tap in this place. (See diagram of piping.)

results. By this time I had discovered that the megaspores were the first to succumb when the plants were chilled, and I also found an occasional sporocarp, among those which had not apparently been blasted, which showed that the megasporangia and oldest set of microsporangia had been killed, but the younger microsporangia were in a healthy state (*fig. 13, b, c*). So far as I could determine, these sporocarps were in a fair way to reach maturity, notwithstanding the fact that the megaspores were all dead. I then shortened the time of application of the spray, in order to determine the shortest period which would kill the megaspores and yet not blast the entire sporocarp. This I found to be no easy task, as it is not possible to determine exactly, from external appearance, the stage of development of the sporogenous tissue. I was able finally to be reasonably sure from

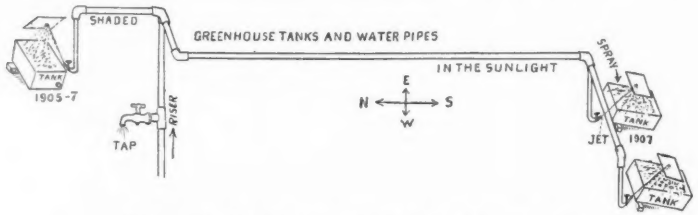

Diagram of piping and position of tanks in greenhouse

their size and thickness when sporocarps had reached the mother cell stage. In such sporocarps, under the conditions of this experiment, the application of the cold spray day and night for 48–60 hours would not cause the entire sporocarp to damp off, but most of the sporocarps when sectioned showed only traces of megaspores in the sporangium. It was in these sporocarps that I got such a great variety of forms of microspores (*figs. 15–22*).

The sporocarps treated less than 48 hours generally developed normally, while those treated for a longer period than 60 hours generally damped off. I was able to treat plants in August and September 1907, in the south end of the greenhouse, in stronger light and at a higher temperature (see table) for a much longer time (76–96 hours) before blasting occurred. Also in the same year I was able to grow abundant and normal sporocarps in tanks out of doors, at temperatures shown in the table, in as much as 20^{cm} of water. At the same

time, plants in the same tank, in 8 to 12^{cm} of water, at the same temperature but shielded from the direct rays of the sun, either blasted completely or showed more or less abortion among the megaspores and occasionally enlargement of some of the microspores. It is evident, therefore, that the plants are dependent on strong light and high temperature in order to mature perfect sporocarps, and that water at any depth less than 20^{cm} need not cause blasting unless it greatly lowers the temperature or reduces the light below a certain minimum.

Discussion

A close inspection of this process of blasting brought to light the important fact that under the conditions of growth to which these plants were subjected the megaspore was the first to show signs of abortion. It might be added that through a long list of experiments on the younger stages of sporocarps this was the invariable rule. Unfavorable conditions of growth, whether brought about by depth of water, lack of light, low temperature, or drought, without exception always affected the megaspores more than the microspores. Later experiments revealed the fact that in the more mature sporocarps this was not the case. Hard conditions brought on after the megaspores are well formed, but about the time the microspores are in the mother cell stage, will produce abortion among the microspores. The critical period in the life of the spore, whether microspore or megaspore, seems to be from the time when the mother cell comes into the synapsis stage until the tetrads are well on the way to becoming spores. This also agrees with Miss PFEIFFER'S observations on Azolla (**20**). However, it proved to be much easier to arrest the development of the megaspores than to affect the microspores in this way, and when abortion did occur it was much more nearly universal in the case of the megaspores than in that of the microspores.

Excellent examples were secured showing sporocarps without a single megaspore, while I was never able to secure the complete blasting or abortion of the microspores alone. Many experiments were made in which both were completely blasted, but if either survived it was always the microspores, though sometimes only a small number. It was also noticeable that when the sporocarps were subjected to such treatment as to blast the megaspores in their early

stages, sometimes, when the plants were afterward placed under the best of growing conditions, only part of the microspores survived the change. The surviving ones would then respond with vigor, becoming sometimes three times the usual size for the normal microspores (*figs. 26, 28*). It was noticed also that when a sporocarp, whose oldest sporangia were just forming tetrads, was subjected to a spray of cold water for several days, the megaspores would abort, and the microspores, which at this time would be just coming into the mother cell stage, would not undergo the reduction division while under the spray, but would continue to grow until they became much larger than the ordinary microspores, assuming at the same time many unsymmetrical and unusual shapes (*figs. 15–22*). When these were placed later in good growing conditions, both reduction divisions occurred without the formation of more than mere traces of walls (*fig. 14, c*), but the entire wall of the mother cell became much heavier. Repeated attempts at germination under the most favorable conditions have so far failed to demonstrate what these spores might become ultimately. The failure to germinate seems to find its explanation, not in any want of vitality of the cell, but rather in the rigidity and continuity of the surrounding wall. As is well known, the heavy walls of both megaspores and microspores are interrupted at the papilla, which has only a light membranous covering, due to the presence of radiating protoplasmic strands (figured in SACHS **22** for *Pilularia globulifera*) that hold the four spores in close proximity until the wall is well developed over the free surfaces. The tapetal layer always incloses the tetrad, but forms a perinium only where it comes in contact with the cell membrane of the functioning spore. It is prevented from touching this wall at the papilla by the other three members of the tetrad (*figs. 6–10*), yet the perinium will not form around an aborting spore. The microspores separate earlier than the megaspores, hence the papilla is less evident in them than in the megaspores where the abortive members of the tetrad may not be cast off at all, often remaining until germination has begun. When the tetrad divisions are delayed, there is not a corresponding delay in wall formation, which goes forward at such a rate that when the divisions occur the wall is too strong to disrupt, though in the second division evanescent cell plates are sometimes evident (*fig. 14, c*).

As there is nothing to interfere, the wall is laid down continuously over the entire surface of the cell, being thicker than the ordinary microspore wall, with no thin places, and absolutely unresponsive to germinative conditions. Cases of delayed or estopped germination due to resistant seed and spore coats are not uncommon, as shown by CROCKER (**15**) in the cocklebur and other plants.

As said before, unfavorable conditions in the early life of the sporocarp produced blasting of the megaspores, and sometimes delayed the reduction divisions in the 16 mother cells. However, unfavorable conditions at a later date permitted a very complex set of results, most of the megaspores blasting, while some of the microspore mother cells would undergo division and separate into spores (*fig. 21*). An interesting condition was obtained by blasting just as the microspore tetrads were formed, and then transferring the plants to the best condition for growth. In this case a few microspores would survive in a few of the sporangia, growing with astonishing vigor, some of them reaching a size quite beyond the ordinary microspores (*figs. 24–27*). We have in this case an intermediate sized spore, sufficiently unlike the microspore to warrant the question whether the abortion of tetrads among the megaspores did not begin in this way. The difference in size between the ordinary microspores and these unusual ones is as great as that shown between the reputed microspores and megaspores of Calamostachys and Sphenophyllum.

Probably the most interesting of all the results were obtained by growing Marsilia in subdued light during the usual time of sporocarp-formation (July and August), and then forcing the plants to develop sporocarps at a later period. I had previously determined that although the plants grew luxuriantly in subdued light, they would not produce sporocarps. On August 15, 1906, plants were moved to the south end of the greenhouse and given the maximum amount of heat and light. My intention in this experiment was to throw all the vigor of the plant into a few sporocarps and then to inhibit further sporocarp-formation by means of a spray of cold water. A few sporocarps appeared by September 10, which was more than two months later than the usual time for sporocarp-formation. These grew with great vigor until the occurrence of exceptionally cool and cloudy weather, which began September 19. After this time no more sporo-

carps appeared, and all the younger ones, already in evidence, began to blast, although I did not use the spray. Sections of these showed, as usual, that the megasporangia were the first to succumb to the unfavorable conditions. Sections of the older ones showed a condition which I had not seen before, namely that the blasting was much more general in the microsporangia, and that in many of the oldest sets of microsporangia megaspores were developing (*figs. 32–37*); while in a few microsporangia abortion had proceeded until only one microspore, which was 8–16 times larger than the ordinary ones, remained (*figs. 28, 31*). The few remaining sporocarps were carefully grown to maturity, and on dissection showed a number of sori containing two megaspores instead of one, as I had found in all the cases thus far noted. That these secondary forms (called secondary because of forming at a later date than the original or primary megaspores, and also because of being smaller; *figs. 32–43*) were megaspores there could be no doubt. Every distinguishing character peculiar to the megaspore was present, such as size, shape, thickness of wall, position and shape of nucleus (which is always apical and meniscoid), and manner of vacuolation. There could be no doubt that these forms, though occurring in microsporangia, were far beyond any microspore in special characters; but they still retained some undoubted microspore characters; such as an approach to the general spherical form, the long axes of the smaller (intermediate) size being proportionally shorter than those of the primary megaspores (*figs. 38–43*). So far I have failed to induce germination in any of these induced forms.

A number of the enlarged microspores from this culture were sectioned and showed certain characters belonging to megaspores. The ordinary microspore nucleus is spherical and central, while that of the megaspore is generally meniscoid and apical. It was easy to secure transition forms of nuclei among these microspores, which ranged from spherical, central ones in the case of the smaller spores, through a series of spheroidal, parietally placed nuclei in larger spores (*fig. 30*), until in the largest sizes, which were also more vacuolated, there was always found a meniscoid nucleus in the apical region of the slightly elongated spore (*fig. 31*).

The microsporangium represented in *figs. 28, 29* shows several

functionless tetrads, which, while now distorted and shriveled, must have drawn heavily upon the supply of nutrition. This may explain why the largest microspores, while acquiring certain megaspore characters, were not able to attain more fully to megaspore proportions. In examining the sporangia that contained secondary megaspores, I was never able to find these large shriveled tetrads. This shows that all the tetrads must have aborted during the early stages of development, thus permitting the whole of the nutrition to be concentrated in this one spore, as is the usual case in primary megasporangia. *Fig. 36* is unusual for secondary megaspores, and shows in addition to the successful spore a single member in each of the three tetrads slightly enlarging. *Fig. 37* is more usual, and shows five tetrads which aborted much earlier. Two cultures were grown in 1907 under conditions similar to those just described, and the same results were obtained. Occasionally in all of these cultures I found megasporangia containing well-developed microspores (*figs. 2, 33, 35, 37*). In ordinary plants these were always found on the periphery of the sporogenous area and appeared to be related to the food supply. Scant nutrition may cause the sporogenous tissue in some megasporangia to revert to the probable ancient homosporous condition.

This production of secondary megaspores I believe to be as closely related to nutrition as is the formation of primary megaspores. In numerous cultures I was able to observe that the formation of megaspores is possible only when the plants are well nourished. In the cultures in which I secured secondary megaspores, I always had long and strong rhizomes and allowed only a few sporocarps to form just at the end of or after the fruiting season. Then by cutting off all later sporocarps I was able to throw all the energies of the plant into a very few. By this means I was able to secure not only the most marked abortion and enlargement of the microspores, but also the formation of secondary megaspores, which, since they occur in microsporangia, would undoubtedly have developed, under ordinary conditions, as microspores.

In a plant so plastic as Marsilia, it is not difficult for one to conceive how the heterosporous habit may have become fixed. If excessive nutrition will now cause certain microsporangia to develop megaspores, and excessively hard conditions will cause the plant to

revert to the homosporous habit, it would seem reasonable to conclude that the plant had attained to heterospory gradually, a few of the better nourished sporangia each developing a few spores slightly larger than the rest. Other things being equal, these spores in germinating would naturally develop the larger prothallia, because of a larger amount of food material within the spore. We might expect that these prothallia would bear archegonia. Thus the determination of the sex of the prothallia would be at once shifted from dependence on the chance external conditions after the spore germinates, as found in the homosporous ferns, to dependence on nutritive conditions while the spore is being formed. This, it appears to me, must have been the process by which heterospory made its appearance in Marsilia.

I should also state that megaspores were noticed which were packed unusually full of starch grains (*figs. 44, 45*). These forms were larger than any megaspores that I have seen and had developed no perinium. I was not able to observe stages in their development nor to account for the phenomenon.

Theoretical discussion

If the doctrine of recapitulation be applied here, we may say that Marsilia can be so manipulated as to show in the development of its sporocarps the various stages in the production of complete heterospory, through the abortion of many spores and the special nutrition of a few.

We know that the heterospory described by WILLIAMSON and SCOTT in such forms as *Calamostachys Casheana* began in a very modest way, the diameter of the megaspores being not more than three times that of the microspores in the most extreme cases. We are also told that in the genus Calamostachys, *C. Binneyana* is homosporous and *C. Casheana* is heterosporous; but even in *C. Binneyana* abortive spores are found, and the remaining spores are larger in proportion as fewer develop in each tetrad, the diameter being fully twice as great when only one develops as when three remain.

WILLIAMSON and SCOTT point out that in *C. Casheana*, also, abortion is evident, but is confined to the megasporangia; in fact, their figures show no abortion in the microsporangia. They conclude, therefore, that heterospory was reached by the abortion of the

spores in certain sporangia; then better nutrition caused the excessive development of the survivors at the expense of the aborting ones; and this process continued until specialized spores (megaspores) were formed.

Heterospory, as found in Calamostachys, is in its incipiency. We see its very beginnings in *C. Binneyana*, where all the sporangia show a tendency toward it by the promiscuous abortion of some members of all the tetrads, with the corresponding enlargement of the survivors. In *C. Casheana* we see it feebly established, abortion being confined to certain sporangia, yet fully three-fourths of them showing abortive spores quite variable in size.

In Marsilia, in ordinary plants, heterospory is much more firmly established, abortion being confined to not more than one-fifth or one-sixth of the sporangia, and at maturity the megaspores are very nearly equal in size, and many times larger than the microspores. But when by any means the fertility of these megaspores is destroyed, there is still a very large number of potential spores in the original homosporous condition and sufficiently young to respond to the stimulus given them by the added nutrition which should have gone toward maturing the megaspores. We then see the plant repeating its probable ancient habit of developing some of the spores to greater size at the expense of the others; and we have repeated in every detail the processes observed in *C. Binneyana*, which is homosporous. Again, well-nourished plants, allowed to produce only a limited number of sporocarps, carry this abortion and enlargement still farther, and a few sporangia produce still larger spores, varying as much in size as in *C. Casheana*, which is heterosporous. *Figs. 32–43* show that in many sporangia abortion and enlargement continue much farther than in *C. Casheana*, in that an intermediate sized megaspore is produced. This adds more evidence as to the plasticity of the plant and the manner in which heterospory may have originated in it.

Summary

1. It is possible by means of a spray of cold water to kill the megaspores of Marsilia, which occur only in the oldest sporangia, and then by putting the plant under good conditions to mature sporocarps without megaspores.

2. The greatest variations occur when the megaspores and the oldest microspores are blasted, and when strong plants develop a few sporocarps out of season.

3. Enlargement does not appear among the microspores when less than half the spores abort, and the surviving spores are larger the greater the amount of abortion.

4. The mother cells may be checked in their development till the tapetal nuclei completely invest them. A perinium will then form around the mother cell wall, which invests the four young spores. In such cases the sporangium invariably contains sixteen large forms each containing four nuclei. At other times, when growth is less checked, the spores are more or less completely free and show great variation in size and shape.

5. The contest for supremacy among the young megaspores of each sporangium is very evident, many of them assuming considerable proportions, but one, centrally located, invariably secures the ascendency. Sometimes the contest is very close between two or more members of the same tetrad. Very often the surviving member will carry attached to its papilla the aborted members even to germination.

6. The enlarged microspores vary in size, the largest being 8–16 times the size of the ordinary ones; the position of the nucleus changing from a central (usual) to an apical one, as in the megaspores. As vacuolation is more extensive, the shape of the nucleus also varies from the usual spherical form to the oval, and finally in the largest to the meniscus shape, as in the megaspore.

7. In extreme cases of abortion in the microsporangia only one spore survives, which is about 16 times as large as the normal microspore. The aborted tetrads remain as in the megasporangium, but are much larger and better developed, thus showing a sharper and more prolonged contest for supremacy.

8. In plants kept from sporocarp-formation until September 10, many microsporangia developed secondary megaspores, so called because they are formed after the first or primary ones. Such megaspores are intermediate in size and are also more nearly the spherical shape of the microspores.

9. A few cases were noted in which the megaspores did not

develop a perinium, but enlarged considerably and became gorged with starch.

10. In the normal plants, and in all cultures, a close examination reveals a homosporous tendency, shown by the formation of microspores in the megasporangia, especially in those most distant from the nutritive supply.

11. Marsilia may be made to repeat, under culture, all the phases in the development of heterospory reported by WILLIAMSON and SCOTT for *Calamostachys Binneyana* and *C. Casheana*, and in addition to produce a megaspore of intermediate size.

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EXPLANATION OF PLATES III–VI

All drawings were made with a Spencer camera lucida. In all drawings showing nuclear detail a Zeiss 2^{mm} apochromatic objective with compensating oculars was used. The drawings and photomicrographs were then reduced to the magnifications indicated in the explanation of figures.

PLATE III

FIG. 1.—Diagrammatic sectional view of sporocarp; *A*, *B*, *C*, *D* designate planes; *C'*, soral pad attached to vascular bundle and subtending a single sorus. ×7.

FIG. 2.—Portion of a section showing three sporangia of the oldest series (right) containing megaspores, and one (left) containing microspores. ×40.

FIG. 3—Young sporangium, showing 8 of the 16 mother cells in synapsis, just as the tapetum begins to form the plasmodium. ×292.

FIG. 4.—Older sporangium, showing tetrads with the tapetal plasmodium dispersed among them. ×292.

FIG. 5.—First stage in which one can determine that the sporangium is to contain megaspores; several spores are enlarging, but the mass of tapetal nuclei is collected about the larger one in the center. ×292.

FIGS. 6–10.—Showing the competition among the members of the tetrad and the persistence of the three aborting members through the various stages of megaspore formation; they are often evident even after germination, as shown in *fig. 10a*. *Figs. 6, 7, 8, 9*, ×300; *10b*, ×85.

PLATE IV

FIG. 11.—Photomicrograph showing the aborted members of the tetrad still attached to the papilla of mature megaspore. ×85.

FIG. 12.—Diagrammatic representation of fruiting rhizomes, showing the effect of varying the depth of water when the plants are in such subdued light as to be just able to produce sporocarps; *b*, blasting; *m*, maturing.

FIG. 13.—Condition of various sporangia of a sorus during the process of blasting; *a*, old aborted microsporangium, megasporangium in the center; *b*, *c*, young microsporangia from same sorus.

FIG. 14.—*a*, normal microsporangium; *b*, sporangium in which the 16 mother cells have not formed walls during the tetrad divisions; *c*, same in section; *d*, sporangium with extensive abortion, a few of the surviving spores enlarged.

FIG. 15.—Portion of a section of a sporocarp in which all the megaspores have aborted, as shown by the empty megasporangia, *m;* in the microsporangia no walls have been formed in the tetrad divisions, so that each sporangium contains but 16 enlarged and irregular bodies instead of the usual number of 64 spherical spores. ×55.

FIGS. 16, 17.—Single entire sporangia, showing the 16 bodies as in *figs. 14b*, and *15*. ×105.

FIG. 18.—Three sections, *a*, *b*, *c*, of various bodies from sporangia as in *figs. 16, 17*, showing nuclear structure and behavior. ×400.

PLATE V

FIGS. 19, 20.—Similar to *fig. 18*; forms more irregular. ×400.

FIG. 21.—Microspores resulting from tetrads which have had varying success in forming walls during the second division, due to unfavorable conditions of growth. ×133.

FIG. 22.—Nuclear structure and behavior in earlier stages of such microspores. ×400.

FIG. 23.—A normal microsporangium at maturity, containing 64 spores. ×95.

FIGS. 24, 25.—Microsporangia in which many of the microspores have

aborted, showing at maturity a number of normal spores and a single enlarged microspore. ×95.

FIG. 26.—More complete abortion of microspores, with four enlarged. ×95.

FIG. 27.—Same more highly magnified.

PLATE VI

FIG. 28.—Microsporangium showing a few remaining but abortive tetrads, and only one (much enlarged) microspore. ×95.

FIG. 29.—Same more highly magnified.

FIGS. 30, 31.—Sectional views showing internal and external transitions from the ordinary spherical microspore, which has a central nucleus and no vacuolation, toward the megaspore form and condition; *fig. 30* shows an enlarged, spherical, but vacuolated spore, with spheroidal nucleus laterally placed; *fig. 31*, a more enlarged, spheroidal spore, more vacuolated, with meniscoid nucleus apically placed. ×180.

FIG. 32.—Section of sporocarp showing primary (*p*) and secondary (*s*) megaspores, as determined by age, position, size, and stage of wall formation. ×33.

FIG. 33.—Same showing three primary (*p*) and two secondary (*s*) megaspores, and one megasporangium, *m*, containing microspores. ×33.

FIG. 34.—Same, showing one central and one lateral section of primary megaspores, *p*, and six secondary megaspores, *s*. ×33.

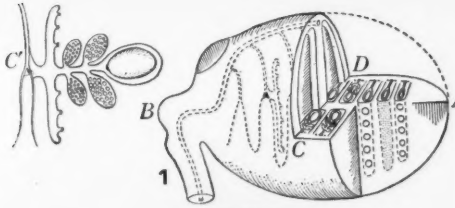
FIG. 35.—Same showing two primary (*p*) and two secondary (*s*) megaspores, and one primary megasporangium, *m*, containing normal and aborting microspores. ×33.

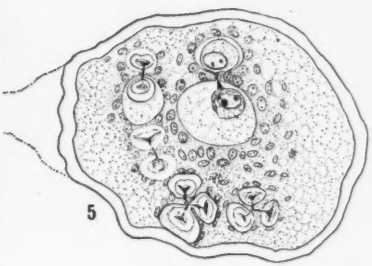
FIG. 36.—Primary and secondary megaspores more highly magnified; the secondary megasporangium showing three sets of tetrads in which one of the spores in each set has enlarged and become vacuolated (not marked in the figure), thus competing sharply with the functioning megaspore, *s*, for supremacy. ×87.

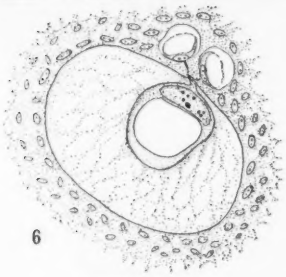
FIG. 37.—Primary megasporangium, *n*, containing microspores nearly mature, one of the oldest microsporangia, *o*, containing a young secondary megaspore, the same age as that shown in *fig. 36;* the microspores are the same age as the primary megaspore in *fig. 36*. ×87.

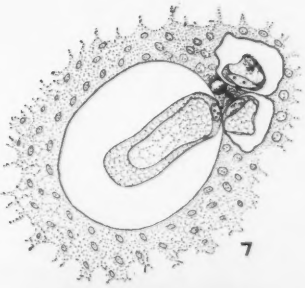
FIGS. 38–43.—Variation in size and shape between the primary (*p*) and the secondary (*s*) megaspores at maturity. ×44.

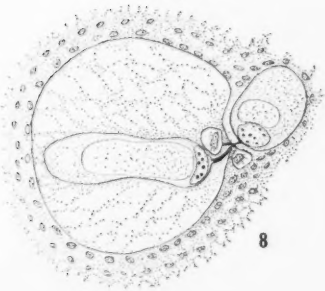
FIGS. 44, 45.—Enlarged megaspores packed full of starch grains, but having no perinium. ×44.

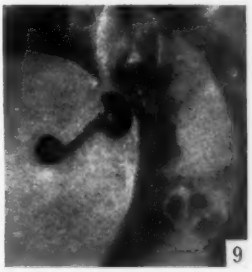


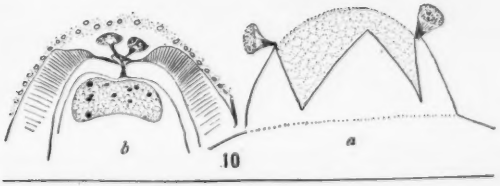




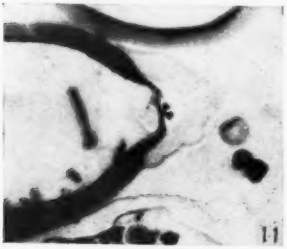








SHATTUCK on MARSILIA



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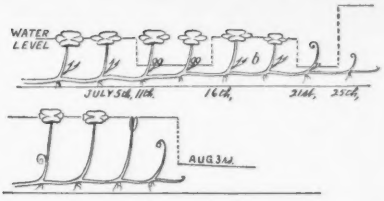

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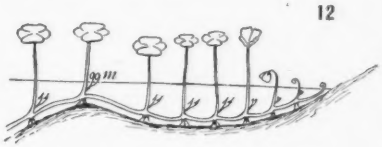

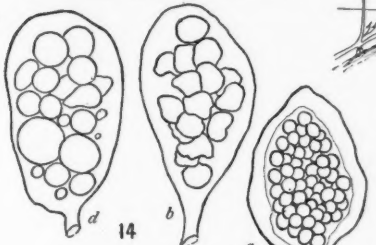

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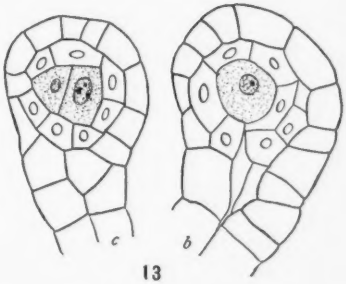

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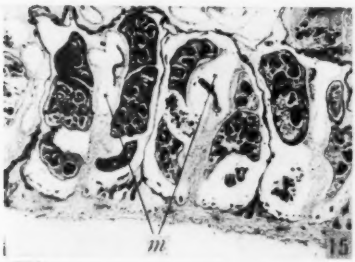

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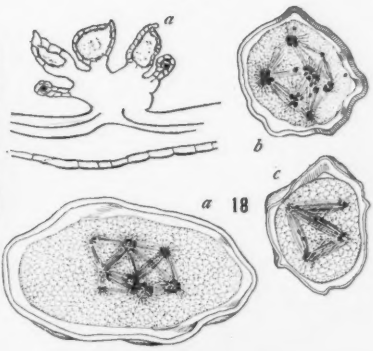

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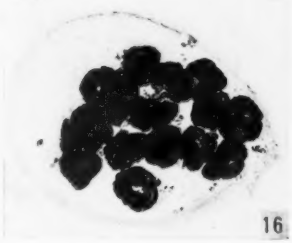

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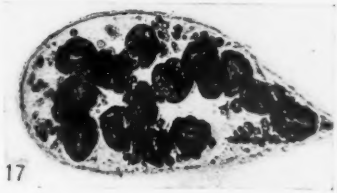

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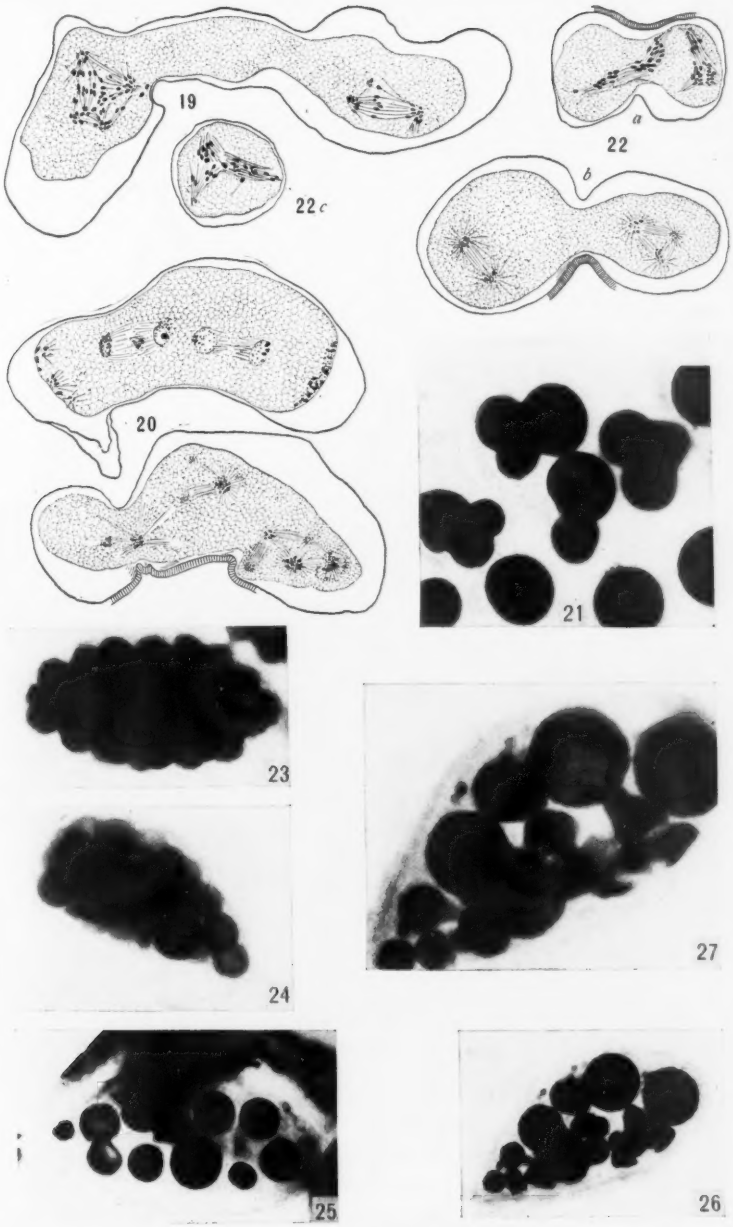
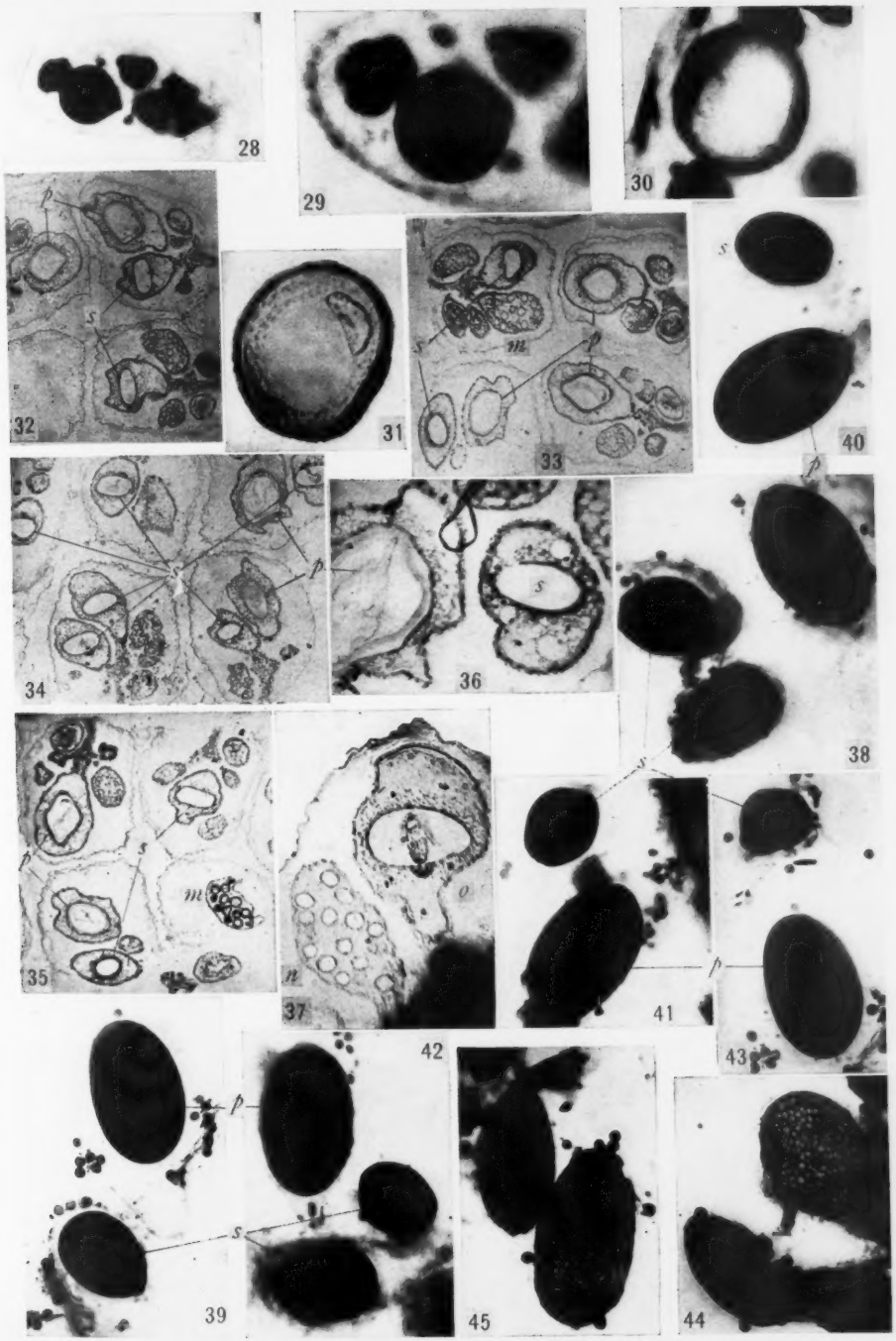
SHATTUCK on MARSILIA



SHATTUCK on MARSILIA



SHATTUCK on MARSILIA

ON THE LACK OF ANTAGONISM BETWEEN CALCIUM VERSUS MAGNESIUM AND ALSO BETWEEN CALCIUM VERSUS SODIUM

CHAS. B. LIPMAN

(WITH TWO FIGURES)

In a former paper,[1] I have shown that many of the facts relating to the toxic and antitoxic effects of salt for animals and plants, as shown by the work of LOEB and OSTERHOUT in particular, hold in a general way with regard to the ammonifying power of *Bacillus subtilis*. In the same paper I also reported the results of experiments which showed that there is in respect to the ammonification by *B. subtilis* no antagonism between calcium and magnesium. As this is so striking an exception to the results obtained by LOEW[2] in his well-known experiments on green plants, it was thought advisable to emphasize the writer's results in a separate article, along with another new and strikingly exceptional case, namely a lack of antagonism between calcium and sodium. The latter case is of great interest, inasmuch as KEARNEY and CAMERON,[3] BENECKE,[4] and OSTERHOUT[5] have all found very pronounced antagonism between sodium and calcium in green plants. OSTERHOUT has also found this to be the case for a mold (Botrytis).[6]

In general, the technic employed in the two sets of experiments reported was as follows: Solutions of chemically pure salts, previously submitted to the flame test, were made up at a concentration of 0.35*m* containing 0.75 per cent. Witte's peptone. Inoculations were made from a 48-hour peptone culture into solutions made up as described in detail below. At the end of the incubation period of 2.5 days at

[1] BOT. GAZETTE **48**:105–125. 1909.

[2] Literature in LOEW, Bull. 18, Div. Veg. Physiol. and Path., U. S. Depart. Agric. 1899; also LOEW and ASO, Bull. Coll. Agric. Tokyo **7**:395. 1907.

[3] Report 71, U. S. Depart. Agric. 1902.

[4] Ber. Deutsch. Bot. Gesells. **25**:322. 1907.

[5] BOT. GAZETTE **42**:127. 1906; **44**:259. 1907; Jahrb. Wiss. Bot. **46**:121. 1908.

[6] Univ. Calif. Publ. Botany **2**:317. 1907.

28–29° C., the culture solutions were transferred to Jena distillation flasks and distilled after adding a slight excess of magnesium oxid. The distillate (about 150^{cc} in bulk) was titrated against tenth normal acid, cochineal being used as the indicator, and the amount of ammonia nitrogen formed thus determined Sterile controls were run on all determinations to allow of an accurate estimation of the ammonia actually formed by *B. subtilis* during the period of incubation.

Series I. $CaCl_2$ versus $MgCl_2$

Solutions of calcium chlorid and magnesium chlorid of 0.35*m* each were made up in bulk to contain 0.75 per cent. peptone. The culture solutions were made and placed in 250^{cc} Erlenmeyer flasks as follows: Flask *A* contained 100^{cc} of the $MgCl_2$ solution made up as noted above; flask *B*, 100^{cc} $MgCl_2$ + 5^{cc} $CaCl_2$; flask *C*, 100^{cc} $MgCl_2$ + 10^{cc} $CaCl_2$; flask *D*, 100^{cc} $MgCl_2$ + 25^{cc} $CaCl_2$; flask *E*, 100^{cc} $MgCl_2$ + 50^{cc} $CaCl_2$; flask *F*, 100^{cc} $MgCl_2$ + 100^{cc} $CaCl_2$. Then beginning at the other end of the series, flask *K* contained 100^{cc} of the $CaCl_2$ solution; flask *J*, 100^{cc} $CaCl_2$ + 5^{cc} $MgCl_2$; flask *I*, 100^{cc} $CaCl_2$ + 10^{cc} $MgCl_2$; flask *H*, 100^{cc} $CaCl_2$ + 25^{cc} $MgCl_2$; flask *G*, 100^{cc} $CaCl_2$ + 50^{cc} $MgCl_2$.

After the solutions were made up, the mixed solutions in each flask amounted to more than 100^{cc}, while the flasks with pure salts (plus peptone) contained only 100^{cc}. In order to make the conditions the same for all the flasks (particularly in respect to the amount of fluid surface exposed to the air), enough liquid was pipetted out of the flasks containing mixed solutions to leave just 100^{cc} in each flask. The flasks were then sterilized in the autoclave at 1.25 atmospheres of pressure for 30 minutes. A 48-hour culture of *B. subtilis* in 1 per cent. peptone was used as inoculating material. By slight shaking the membrane formed at the surface was precipitated to the bottom, and by tilting the flask to one side and carefully setting it down again, one part of the bottom of the flask remained free of membranous material and the liquid above was homogeneous in character. Of this homogeneous liquid 1^{cc} was taken for inoculation in each of the flasks prepared as above described. The table of results follows:

TABLE I

Numbers in first column refer to c.c. of 0.35*m* solutions

Culture solution	Corresponding points on curve	N as NH_3 formed in cultures, in mg
100 $MgCl_2$	A	3.08
100 $MgCl_2$ + 5 $CaCl_2$	B	2.59
100 $MgCl_2$ + 10 $CaCl_2$	C	1.68
100 $MgCl_2$ + 25 $CaCl_2$	D	0.98
100 $MgCl_2$ + 50 $CaCl_2$	E	0.21
100 $MgCl_2$ + 100 $CaCl_2$	F	0.07
50 $MgCl_2$ + 100 $CaCl_2$	G	0.00
25 $MgCl_2$ + 100 $CaCl_2$	H	0.00
10 $MgCl_2$ + 100 $CaCl_2$	I	0.00
5 $MgCl_2$ + 100 $CaCl_2$	J	0.00
100 $CaCl_2$	K	0.49

By an examination of the curve drawn on the basis of table I (*fig. 1*), we are confronted by the very striking instance of no antagonism between the two salts employed. On the contrary, there is a constant increase of the toxic properties of each when the other is added to it in increasing amounts. In this exceptional behavior, so far as I can ascertain, *B. subtilis* (and probably all the ammonifiers) stand alone when their physiological efficiency in such salt mixtures is compared with that of the higher plants and animals. No instance of such behavior on the part of any member of the latter two groups of organisms has come to my notice in reviewing the results of similar researches on animals and the higher plants.

We find among plants the well-known researches of LOEW[7] and his pupils, and later the researches of KEARNEY and CAMERON,[8] which show the strong antagonism between calcium and magnesium.

[7] Bull. 45, Bureau Pl. Ind., U. S. Depart. Agric.; also LOEW and ASO, Bull. Coll. Agric. Tokyo **7**:no. 3. 1907.

[8] Report 71, U. S. Depart. Agric.

The last-named investigators found in their experiments with the white lupine (*Lupinus albus*) and with alfalfa (*Medicago sativa*) that when $CaCl_2$ was added to $MgSO_4$ in about equal proportions the plants exhibited about 160 times the tolerance for the latter salt that they did in solutions of $MgSO_4$ alone. They found further that the antagonism between $CaCl_2$ and $MgCl_2$, though not so great (increasing the tolerance about 40 times), was nevertheless very marked, and

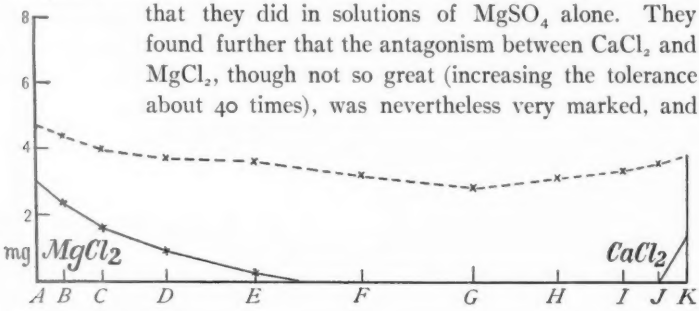


FIG. 1.—The ordinate at *A* represents the amount of ammonia nitrogen in milligrams formed in a pure 0.35*m* solution of $MgCl_2$ containing 0.75 per cent. peptone. The ordinate at *K* represents the amount of ammonia nitrogen formed in a pure $CaCl_2$ solution of the same strength and with the same peptone content. The ordinates at the intermediate points represent the amounts of ammonia nitrogen formed in various combinations of the two salts as indicated in tables I and II. The full line represents results in table I; the broken line represents results in table II.

where $CaSO_4$ replaced $CaCl_2$ the antagonism was very much greater between Ca and Mg than in either of the cases above cited. BENECKE[9] likewise found antagonism between magnesium and calcium in his work with Spirogyra.

An antagonism between $CaCl_2$ and $MgCl_2$, though slight, was found to be none the less definite by LOEB[10] in experiments which showed that sea urchin blastulae and gastrulae would swim about in a mixture of the salts above mentioned for 48 hours, while each salt by itself would immediately prove poisonous at the concentration employed in the combination. Another interesting case in point may be noted in the experiments of the same investigator on Polyorchis,[11] a jellyfish of San Francisco Bay. In a solution of 50^{cc} NaCl + 6^{cc} $MgCl_2$ + 1^{cc} $CaCl_2$, the rhythmical contractions of the margin go

[9] Ber. Deutsch. Bot. Gesells. **25**:322. 1907.

[10] Amer. Jour. Physiol. **3**:327. 1900.

[11] Jour. Biol. Chem. **1**:427. 1906.

on normally, but with a slight increase of $CaCl_2$ the contractions are inhibited, and when 5^{cc} of a $0.375m$ solution of $CaCl_2$ are added, they are completely suppressed. On the other hand, when the margin of the fish, containing the sense organs and the central nervous system, is cut off, $CaCl_2$ exercises a stimulating action on the isolated center of Polyorchis and contractions go on normally; but when $MgCl_2$ is added to the solution in the ratio of 4 parts $MgCl_2$ to 1 part $CaCl_2$, the stimulating action of the $CaCl_2$ is suppressed and contractions cease. In both cases, therefore, there is evidence of a definite antagonism between calcium and magnesium. LILLIE[12] also proved the existence of antagonism between the two salts when he found that the ciliary activity of the larvae of Arenicola would go on normally for some time in a mixture of approximately 4 parts $MgCl_2$ to 1 part $CaCl_2$, whereas it would cease immediately if either of the salts at the same concentration was present alone. The same investigator found in other work that calcium salts inhibit the spontaneous contractility of the swimming plate in ctenophores, when added to solutions made up of 90 parts by volume NaCl and 10 parts $MgCl_2$. Only 2 volumes of $CaCl_2$ of the same concentration as the other salts inhibit movements of the swimming plate in the sodium and magnesium solution above described, while 4 volumes of $CaCl_2$ allow but little spontaneous movement. The latter is entirely inhibited by the addition of 8 volumes of the $CaCl_2$ solution.

I wish to cite only one more case, which emphasizes by contrast most strongly the exceptional results obtained above in experiments with *B. subtilis*, and that is, the remarkable results obtained in a highly ingenious series of experiments recently carried out by MELTZER and AUER[13] on the antagonistic effect of calcium on the inhibitory effect of magnesium. The experiments were carried out on rabbits, and in one case on a monkey, and as a typical instance of the remarkable antagonistic effect between calcium and magnesium may be cited the first experiment of the series, in which about 13^{cc} of an $m/1$ solution of $MgCl_2$ was injected subcutaneously into a rabbit. Less than one-half hour later there was produced general anaesthesia, with all the attending symptoms, and a $0.125m$ solution of

[12] Amer. Jour. Physiol. **5**:56. 1901.

[13] Amer. Jour. Physiol. **21**:403. 1909.

$CaCl_2$ was injected intravenously in the ear vein. When only 2^{cc} had thus been injected, the rabbit was again breathing normally, and when 8^{cc} had been given, the animal sat up and appeared entirely recovered, except for a stiffness in the hind legs.

In these experiments, some of which were even more striking than the one cited, MELTZER and AUER employed, besides the chlorids of calcium and magnesium, the acetate and nitrate of the former, and the acetate, nitrate, and sulfate of the latter, and the same strong antagonism was noted in all cases.

In addition to the confirmation of the results obtained above in my experiments with the same material, one series was also carried out with a culture of *B. subtilis* obtained fror an entirely different source, and the salt solutions made up from a different grade of chemically pure salt. As can be seen from the following table, the results fully

TABLE II

Numbers in first column refer to c.c. of 0.35*m* solutions

Culture solutions	Corresponding points on curve	N as NH_3 formed in cultures, in mg
100 $MgCl_2$	A	4.76
100 $MgCl_2$ + 5 $CaCl_2$	B	4.48
100 $MgCl_2$ + 10 $CaCl_2$	C	4.20
100 $MgCl_2$ + 25 $CaCl_2$	D	3.78
100 $MgCl_2$ + 50 $CaCl_2$	E	3.64
100 $MgCl_2$ + 100 $CaCl_2$	F	3.22
50 $MgCl_2$ + 100 $CaCl_2$	G	3.08
25 $MgCl_2$ + 100 $CaCl_2$	H	3.29
10 $MgCl_2$ + 100 $CaCl_2$	I	3.43
5 $MgCl_2$ + 100 $CaCl_2$	J	3.57
100 $CaCl_2$	K	3.78

confirm those above given, and though the absolute amounts are different, the results are relatively the same (see also *fig. 1*).

It may be of interest to note here that *B. subtilis* from a 24-hour peptone agar slope culture was examined in hanging drops of molecular solutions of calcium chlorid and showed no perceptible ill-effects from the action of the solution. The ciliary movements appeared normal even after 24 hours in the hanging drop. It was noticed, however, that there was little or no division among the bacilli during the 24 hours, and it is likely that the calcium and magnesium salts exercise their toxic effects by inhibiting reproduction, since the ciliary movements seems to go on without interruption. These remarks, however, are based on too meager experimental evidence to partake of anything else than the nature of conjecture, but they serve to indicate a field of most interesting possibilities in research.

Though they are not analogous instances of the lack of antagonism between calcium and magnesium as shown above, it is interesting to note two cases on record, in which the addition of one salt to another made a combination more toxic than either. One case is that of OSTWALD'S[14] experiments on the fresh-water Gammarus, in which it was found that a combination of $MgCl_2$ and NaCl in solution was more toxic to that animal than NaCl in solution alone. The other case is that noted in the experiments of PAUL and KRÖNIG,[15] who found that the value of mercuric sulfate, acetate, and nitrate as disinfectants was enhanced by the addition of small amounts of the chlorids of potassium and sodium; but, on the other hand, the addition of the same chlorids to $HgCl_2$ reduced considerably the disinfecting power of the latter. The first instance is not analogous to my results, because one of the salts used by OSTWALD was different and the experiment was carried out under conditions so totally different that the value of a comparison here is doubtful. In the second instance, as PAUL and KRÖNIG themselves suggest, the increase of toxicity is not necessarily owing to a lack of antagonism between the two salts, but rather to the formation of complex double salts of mercury, characteristic of that element, and therefore this again cannot be compared with the lack of antagonism between calcium and magnesium above noted.

[14] Univ. Calif. Publ. Physiology **2**:163. 1905.

[15] Zeitschr. Hyg. und Infectionskrank. **25**:57. 1897.

Series II. $CaCl_2$ versus NaCl

In this series the solutions were made up in the same way as in the preceding one, and the same concentration of salts and peptone was employed, the only difference being that $CaCl_2$ and NaCl were tested instead of $CaCl_2$ and $MgCl_2$. The incubation was carried out for two and one-half days at 28–29° C., and the distillations and determinations were made as above described. The table of results follows:

TABLE III

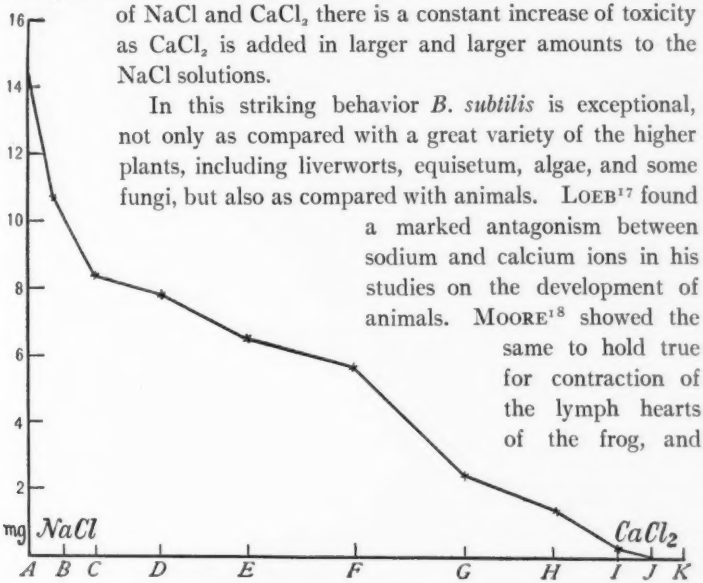
Numbers in first column refer to c.c. of 0.35*m* solutions

Culture solutions	Corresponding points on curve	N as NH_3 formed in cultures, in mg
100 NaCl.....	A	14.48
100 NaCl 5 $CaCl_2$ }	B	10.94
100 NaCl 10 $CaCl_2$ }	C	8.46
100 NaCl 25 $CaCl_2$ }	D	8.18
100 NaCl 50 $CaCl_2$ }	E	6.64
100 NaCl 100 $CaCl_2$ }	F	5.99
50 NaCl 100 $CaCl_2$ }	G	2.54
25 NaCl 100 $CaCl_2$ }	H	1.53
10 NaCl 100 $CaCl_2$ }	I	0.07
5 NaCl 100 $CaCl_2$ }	J	0.00
100 $CaCl_2$.....	K	0.00

In the curve drawn on the basis of the foregoing table (*fig. 2*) we note again the striking instance of the lack of antagonism for *B. subtilis* of two salts which showed a strong antagonism in all experiments on animals and plants thus far carried out. OSTERHOUT[16] showed, for example, that a strong antagonism exists between NaCl and $CaCl_2$ for wheat, and that the curve obtained there was very similar

[16] BOT. GAZETTE **48**:98–104. 1909.

to the curve obtained for the antagonism between KCl and $CaCl_2$. This latter fact is especially interesting, since for *B. subtilis* a strong antagonism is exhibited between KCl and $CaCl_2$, while in the case of NaCl and $CaCl_2$ there is a constant increase of toxicity as $CaCl_2$ is added in larger and larger amounts to the NaCl solutions.



In this striking behavior *B. subtilis* is exceptional, not only as compared with a great variety of the higher plants, including liverworts, equisetum, algae, and some fungi, but also as compared with animals. LOEB[17] found a marked antagonism between sodium and calcium ions in his studies on the development of animals. MOORE[18] showed the same to hold true for contraction of the lymph hearts of the frog, and

FIG. 2.—The ordinate at *A* represents the amount of ammonia nitrogen formed in a pure 0.35*m* solution of NaCl containing 0.75 per cent. peptone. The ordinate at *K* represents the amount of ammonia nitrogen formed in a pure 0.35*m* solution of $CaCl_2$ and with the same content of peptone. The ordinates at the intermediate points represent the amounts of ammonia nitrogen formed in various combinations of the two salts as indicated in table III.

LINGLE[19] also demonstrated that fact in the case of the turtle's heart. In addition to these facts, we have the work of LILLIE[20] to show also that as regards ciliary activity an antagonism between $CaCl_2$ and NaCl was found to exist, and MCCALLUM in his work on cathartics showed the same facts to hold true there.

Many more instances could be cited to show by contrast the strikingly exceptional behavior of *B. subtilis* as regards the two salts

[17] Amer. Jour. Physiol. **3**:328, 383. 1909.

[18] *Ibid.* **5**:87. 1901. [19] *Ibid.* **4**:265. 1900. [20] *Ibid.* **5**:56. 1901.

in question, but only one important case comes to my notice which resembles that of *B. subtilis* just cited, and that is to be found in the work of LOEB with the marine Gammarus, in which he found that the animals die more quickly in a solution of 100^{cc} $0.375m$ NaCl to which 1^{cc} of a $0.375m$ $CaCl_2$ solution was added than in a pure $0.375m$ NaCl solution.

The results set forth in the foregoing pages, as well as those contained in a previous paper, tend to show that in their behavior toward salts bacteria differ in some respects from both plants and animals and occupy a position by themselves. It is evident that mineral fertilizers applied to the soil will not have altogether the same effect on the bacteria as on the higher plants. Further studies in this direction may lead to important practical application.

Summary

1. No antagonism exists between magnesium and calcium. Any combination of the two salts is more toxic than $MgCl_2$ alone for *B. subtilis*.

2. No antagonism exists between sodium and calcium. Any combination of the two salts renders it more poisonous than the NaCl alone for *B. subtilis*.

3. In these two respects, the behavior of *B. subtilis* finds no parallel among plants so far as studied, and scarcely any among animals.

My thanks are due Professor W. J. V. OSTERHOUT for helpful suggestions and criticisms in this work.

LABORATORY OF SOIL BACTERIOLOGY
UNIVERSITY OF CALIFORNIA

BRIEFER ARTICLES

THE MICROSPOROPHYLLS OF GINKGO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 134

(WITH PLATE VII)

This study was undertaken in the first place to determine the origin of the mucilage ducts in the hump of the microsporophyll. As there is a general resemblance among the microsporophylls of Taxus, Torreya, and Ginkgo, and as in Torreya, according to COULTER and LAND[1] (p. 163), the resin ducts are formed from the disorganization of three of the seven sporangia, it seemed possible that in Ginkgo the mucilage ducts might have been formed from abortive sporangia also. As my material showed the development of the strobili and microsporangia, I have included an account of them, so as to give a general history of the development of the microsporophyll. The buds were collected during July, August, and September of 1907 from a tree in Elyria, Ohio, in grounds then owned by Mr. I. W. METCALF. Thanks are due him for the material and also Professor F. O. GROVER of Oberlin College and Mr. FRED STOKEY for collecting.

Development of the strobili

The strobili which will mature pollen the following year are formed in June or early July, but are hidden in buds which cannot be distinguished from leaf buds. In the latter part of July, if the scale leaves are removed from the buds, the strobili can be seen as small papillae in the axils of the scale leaves. Examined with a microscope, strobili of the same bud often show different stages of development, some being short, with the sporophylls just evident as small rounded elevations (*fig. 1*), others being elongated cones with the lower sporophylls beginning to show differentiation into sporogenous part and hump (*fig. 2*). The development of the two sides of the strobilus is often very uneven, due to greater crowding on one side than on the other. At this stage there is no differentiation of tissue in the sporophylls at the top of the strobilus, but in those lower down mucilage ducts are beginning to form in the most external portion of the sporophylls; and in sporophylls at the base the ducts are fully formed and functioning sporogenous tissue is beginning to be evident in the part nearest the axis. Occasionally

[1] COULTER, JOHN M., AND LAND, W. J. G., Gametophytes and embryo of *Torreya taxifolia*. BOT. GAZETTE **39**:161–178. *pls. A, 1–3.* 1905.

the sporangium that will finally function appears in advance of the mucilage ducts, or the two may start together, but generally the ducts are formed before the functioning sporogenous tissue is very evident. By August 8 the sporophylls at the base of the strobilus show the formation of the stalk and the elongation of the functioning sporangium (*fig. 3*), while the sporophylls from the base to half-way up the strobilus have well-developed mucilage ducts. The stalks are formed by an increase in the number of cells as well as by elongation of the cells. By August 20 all the sporophylls have stalks and mucilage ducts, but the functioning sporogenous tissue is conspicuous only in the lower sporophylls. Early in September (*fig. 4*) the strobili have reached a length of 3^{mm}, the sporophylls have the typical shape, the mucilage ducts are enormous, and the cells of the sporogenous mass are separating for the mother cell stage, which the oldest sporangia reach before winter, according to SPRECHER[2] (p. 156). Material that I collected in April shows some sporangia in the mother cell stage and others with tetrads formed.

Development of the functioning sporangium

The early development of the functioning sporangium is probably like that of the cycads,[3] though I have not yet been able to determine that the archesporium starts as a single cell, but stages like that in *fig. 5* probably indicate that a single cell has divided anticlinally and then periclinally. Sometimes the order seems to be reversed. The outer cells are the primary wall cells, giving rise to several layers of wall cells with scanty cytoplasm; the inner are the primary sporogenous cells, by further division producing a large mass of sporogenous cells with dense cytoplasm, larger nuclei with heavily staining chromatin, and frequently conspicuous nucleoli. In the mature sporangium the hypodermal layer of cells and the layer below it have thickening bands in the walls, and in the upper part of the sporangium near the mucilage duct the walls of deeper cells are also thickened. In this respect the microsporangium of Ginkgo differs from the microsporangium of all other gymnosperms and is like that of angiosperms.

The tapetum seems to come entirely from the sporogenous mass.

Development of the mucilage ducts

The upper, sterile part of the sporophyll is developed into a humplike projection called by ČELAKOVSKÝ[4] (p. 283) a crest (*crista*). In this are

[2] SPRECHER, ANDREAS, Le *Ginkgo biloba* L. pp. 206. *figs. 225*. Genève. 1907.

[3] SMITH, FRANCES GRACE, Morphology of the trunk and development of the microsporangium of cycads. BOT. GAZETTE **43**:187–204. *pl. 10*. 1907.

[4] ČELAKOVSKÝ, L. J., Die Vermehrung der Sporangium von *Ginkgo biloba* L. Oesterr. Bot. Zeitschrift **50**:276–283. 1900.

large mucilage ducts, cells containing calcium oxalate, and epidermal cells containing tannin. The mucilage ducts begin development like sporangia, and for some time look like the functioning sporogenous tissue in the lower part of the sporophyll. Several hypodermal cells are differentiated from the rest by their greater size and their more vacuolate contents (*fig. 6*). Each of the cells divides into an outer and an inner cell, suggesting the primary wall cell and primary sporogenous cell of the cycads (*fig. 7*). The inner cells by further division produce a mass of deeply staining tissue beneath several layers of cells with scanty cytoplasm formed by the division of the outer cells (*fig. 8*). As soon as this stage is reached the walls of the central mass of cells begin to disintegrate, probably by becoming mucilaginous (*fig. 9*), as soon the nuclei appear to be floating in a cavity filled with mucilage. Then the nuclei also disintegrate, and some of them with the remains of their surrounding cytoplasm are flattened against the wall of the widening cavity, forming a tapetum-like layer (*fig. 10*). Finally the whole central mass is replaced by a mucilage cavity.

Discussion of the nature of the hump

The sporophylls of Ginkgo resemble those of Taxus and Torreya. Taxus has a group of sporangia, five or more in number, surrounding a central axis. Torreya has four sporangia on one side of the axis, but in the primary sporogenous cell stage seven sporangia appear; three of these disorganize, being replaced by a single large resin cavity, leaving only four to come to maturity. Ginkgo has two sporophylls on one side of the axis, surmounted by the conspicuous hump containing mucilage ducts. The development of the mucilage ducts from what appear to be abortive sporangia seems to indicate that the microsporophylls of Ginkgo may have come from a peltate type like that of Taxus. The fact that the sporophylls of living Ginkgo sometimes, and of fossil Ginkgoales as Baiera regularly, bear more than two microsporangia is additional evidence in favor of this view.

The case would be quite clear if evidence could stop with the hump, but in examining the mucilage ducts in the leaves, I find that they also at first look like sporogenous tissue (*fig. 11*). So there are three lines of development that resemble one another: the functioning sporogenous tissue, the mucilage ducts in the hump of the sporophyll, and the mucilage ducts in the leaves; all have the same early history, starting with one or more cells which divide periclinally into outer and inner cells, the inner by further division forming a deeply staining mass, the outer forming several layers of wall cells. The tissue formed in the functioning sporangium we know is sporogenous as it later produces spores; the tissue in the hump of the sporo-

phyll that forms mucilage ducts, we think is sporogenous from analogy with Torreya and Taxus; but as to the mucilage ducts in the leaves we have no evidence that the tissue is sporogenous. Can we say that the ducts in the leaves, though they look like abortive sporangia, are not abortive sporangia, while the ducts in the hump having the same appearance are really abortive sporangia? On the other hand, does the presumption that the ducts in the leaves are not abortive sporangia forbid entirely the view that the ducts in the hump are abortive sporangia?

It seems reasonable to conclude, if the resin cavity of Torreya originates in abortive sporangia, that the mucilage ducts of Ginkgo also owe their origin to abortive sporangia.

Summary

1. The development of the strobilus proceeds from the bottom toward the top, the oldest sporophylls being at the base.

2. The development of the functioning microsporangium is probably like that of the cycads.

3. The development of the mucilage ducts in the hump of the microsporophylls is in general like that of sporogenous tissue.

4. This origin of the mucilage ducts in the hump may indicate that the microsporophylls of Ginkgo have come from a peltate type like the microsporophylls of Taxus.

I wish to acknowledge my indebtedness to Professor CHARLES J. CHAMBERLAIN, by whose suggestion and under whose direction the work was done.—ANNA M. STARR, *The University of Chicago.*

EXPLANATION OF PLATE VII

FIG. 1.—Longitudinal section of young strobilus, with sporophylls beginning to be evident; August 7. ×20.

FIG. 2.—Longitudinal section of older strobilus, with mucilage ducts (*d*) and sporogenous tissue (*s*) starting at the base; July 29. ×20.

FIG. 3.—Longitudinal section of strobilus older than *fig. 2*, showing development proceeding toward the top; August 7. ×20.

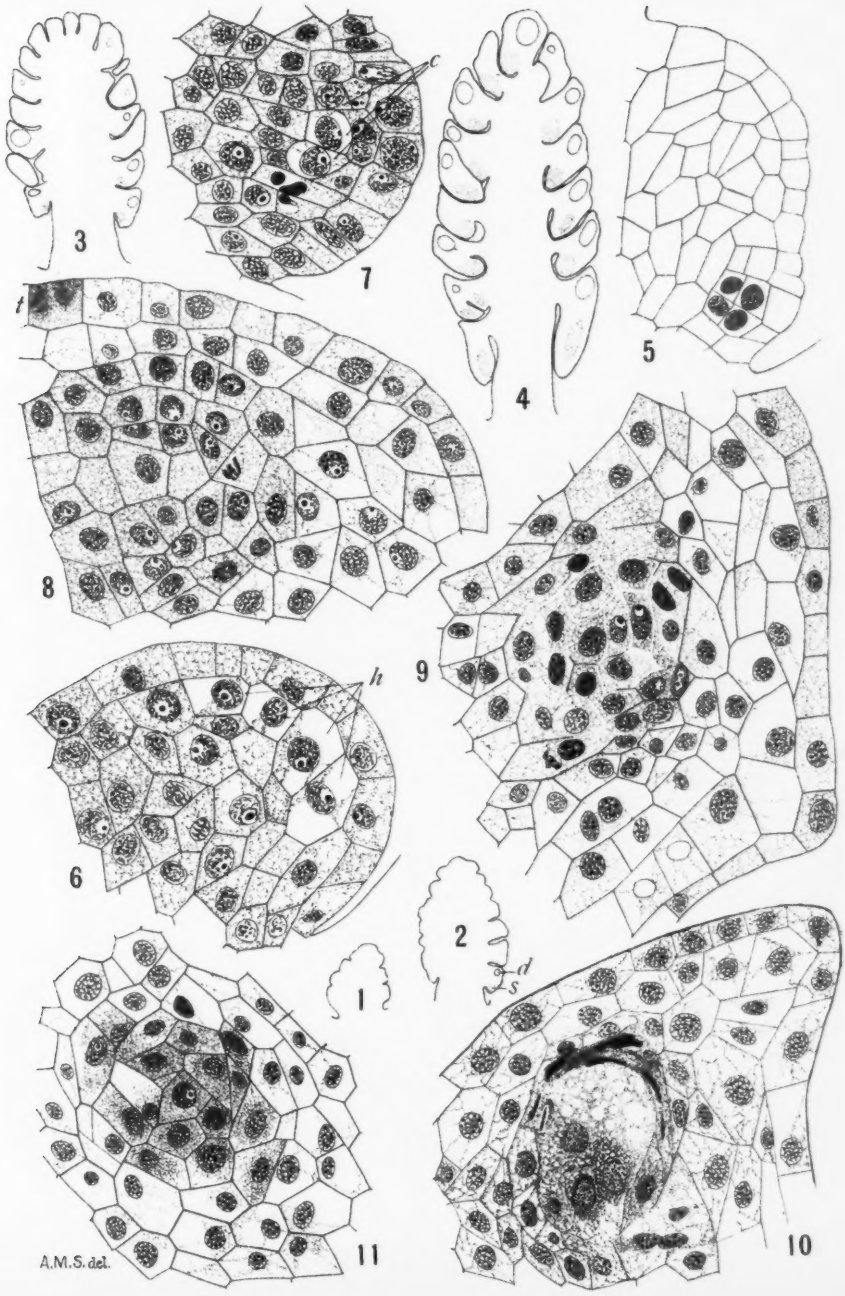
FIG. 4.—Longitudinal section of strobilus with mucilage ducts in all the sporophylls and the sporangia in the mother cell stage; September 2. ×20.

FIG. 5.—Longitudinal section of young sporophyll, showing four cells that have probably come from a single archesporial cell. ×485.

FIG. 6.—Longitudinal section of the hump of a young sporophyll showing a hypodermal plate of four cells (*h*) strongly differentiated. ×485.

FIG. 7.—Longitudinal section of the same region of another sporophyll with three hypodermal cells (*c*) that have divided periclinally. ×485.

FIG. 8.—Longitudinal section of the same region of an older sporophyll


3
7
c
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5
t
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h
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A.M.S. del.

showing differentiation into wall cells and a central mass of cells like sporogenous tissue. ×485.

FIG. 9.—The walls of the cells of the central mass are becoming mucilaginous and some of the nuclei are disintegrating. ×485.

FIG. 10.—The mucilage cavity is formed, some nuclei still floating in the fluid contents, others being flattened against the wall. ×485.

FIG. 11.—Section of an early stage of a mucilage duct in a leaf, showing cells resembling sporogenous tissue. ×485.

ALTERNATION OF GENERATIONS AND SEXUALITY IN *DICTYOTA DICHOTOMA*

After the failure of many attempts made during the past three summers to raise *Dictyota dichotoma* (Huds.) Lamour. from the spores and fertilized eggs to maturity, I have succeeded in raising nearly 100 plants to a fruiting condition. Since all attempts to raise plants to maturity in the laboratory have failed so far, the cultures were started in the laboratory and then transferred to the harbor. The method employed was as follows. Several days before the time for the maturing of the crop of sexual cells, when therefore no eggs were present in the water to serve as a possible source of contamination, two good fruiting tetrasporic plants were gathered late in the afternoon and placed in two jars of sea water, one in each jar. Since the spores will not attach themselves to glass, oyster shells which had lain on land for several years were placed in these jars. On the following day many tetraspores had been liberated and had attached themselves to the shells. The parent tetrasporic plants were then removed.

On the day when eggs and spermatozoids were mature, several sexual plants were gathered late in the afternoon and placed in a jar of sea water with oyster shells in the bottom, as before. Abundant liberation of eggs and spermatozoids occurred the next morning, as usual, and the parent sexual plants were then removed.

Three cultures were thus obtained, one containing fertilized eggs, and the other two containing tetraspores, each of the latter containing the spores from a single plant. These cultures were placed in front of a north window of the laboratory.

After about two weeks, when the young plants had reached a height of $1.5–2^{mm},^{5}$ one shell bearing many young plants was removed from each of these jars and suspended between posts about 30^{cm} below low water in a favorable situation in the harbor, and all plants of Dictyota in the immediate vicinity were removed.

[5] This rate of growth is probably less than normal, since plants of Dictyota grow much more slowly in cultures than they do under natural conditions.

After about two months, on the day when general liberation of the sexual cells occurred, when therefore all sexual plants could be certainly distinguished, these three shells were brought to the laboratory and examined. Each shell bore many plants of Dictyota 1.2–15^{cm} high.[6] Every plant was removed and examined under the microscope. The shell to which fertilized eggs had attached themselves bore 33 plants 2.5–15^{cm} high, all fruiting and all tetrasporic, as was shown by the presence of tetraspores or tetraspore mother cells on every plant. The two shells to which tetraspores had attached themselves bore 64 plants 2.2–13.75^{cm} high, all fruiting, and all sexual, and a few plants 1.2–2.5^{cm} high which were sterile. In handling these cultures many fragments were broken off; these were all examined with the microscope in order to be certain that no plant from any culture was overlooked. The evidence from these fragments agreed entirely with that given above for whole plants.

Since these cultures were placed in the open harbor, the possibility of contamination by spores or eggs floating in the water must be considered. In fact, a few specimens of several other species of algae and many animals (ascidians, worms, molluscs, hydroids, etc.) did attach themselves to the shells. However, the fact that all the 33 plants on the shell to which fertilized eggs had attached themselves were tetrasporic, and all the 64 fruiting plants on the shells to which tetraspores had attached themselves were sexual, seems convincing evidence that no contamination by Dictyota spores or eggs occurred. Thus the belief in the alternation of tetrasporic and sexual generations in *Dictyota dichotoma*, previously based on cytological evidence alone, seems proven by the results of these cultures.

As was noted above, each of the cultures of sexual plants was produced from the tetraspores of a single plant. One of these cultures bore 17 plants (14 females and 3 males), the other bore 47 plants (26 females and 21 males). The tetraspores of a single plant are thus seen to produce plants of both sexes. From the proportion of male and female in the latter culture and from the fact that plants gathered in the harbor at random show males and females in nearly equal numbers, the possibility is suggested that half of the tetraspores produce males and the other half produce females. Material has been preserved for a cytological study of this plant, to discover whether sex determinants occur in this species. These and other results bearing on the process of reproduction in Dictyota will be given in a subsequent article.

This work has been done at the laboratory of the Bureau of Fisheries

[6] This rate of growth is much less than that observed in Dictyota under other conditions.

at Beaufort, N. C. I am indebted to Hon. GEORGE M. BOWERS, U. S. Fish Commissioner, for the privilege of working in this laboratory, and to the director, Mr. HENRY D. ALLER, for many courtesies extended to me during this investigation.

Summary

Plants of *Dictyota dichotoma* raised from fertilized eggs gave 33 tetrasporic plants and no sexual ones. Plants raised from tetraspores gave 64 sexual plants and no tetrasporic ones.

The tetraspores of a single plant produced both male and female plants, in one case in about equal numbers.—W. D. HOYT, *The Johns Hopkins University.*

MICROTECHNIQUE FOR WOODY STRUCTURES

In the preparation of thin sections (5 μ or less) of hard tissues the celloidin method has been largely used with excellent results. The method as originally described by PLOWMAN[7] has been modified during the last few years at the laboratories of plant morphology of Harvard University, to meet the demands of work with special classes of hard tissues.

In reply to numerous inquiries as to the best method of preparing thin sections of woody tissues (trees and shrubs), and as to the advisability of using the celloidin technique, the following methods of treatment used in preparing slides for photomicrography, the study of wood structure, and instruction in wood technology are described.

1. SELECTION OF MATERIAL.—In working with old and thoroughly dried material, the blocks for treatment should be cut from the interior of the piece and as far from the finely checked outer surface as possible. Green material and sapwood are often preferable in working with the woods of gymnosperms, especially with the pines, in order to prevent shredding of the transverse sections and tearing-out of the resin canals. The blocks should be cut in such a manner that the faces represent sections which are as nearly transverse, radial, and tangential as possible. In working with soft woods, larger blocks may be used to advantage than with hard woods, and in the case of extremely hard woods the transverse face particularly must be trimmed down to small dimensions.

2. BOILING.—The blocks should be given a very thorough boiling in water to drive out the air and allow the hydrofluoric acid, used in the next step of the process, to penetrate to all parts of the wood. Repeated boilings and additions of cold water hasten the process of driving out the air.

[7] PLOWMAN, A. B., The celloidin method with hard tissues. BOT. GAZETTE 37:456–461. 1904.

3. DEMINERALIZATION.—It will be found advantagous to use much stronger solutions of hydrofluoric acid with the harder woods. The material should be allowed to remain about two weeks in a solution of one part commercial acid to one part water. In the case of very hard woods, such as live oak, ebony, eucalyptus, etc., pure acid may be used to advantage and the wood soaked two to three weeks. The percentage of solution and length of immersion depend on the hardness of the wood, and should be varied to meet the demands of each species. The acid should be thoroughly washed out, and the material left for several days in a mixture of one part 30 per cent. alcohol to one part glycerin.

4. SECTIONING.—This part of the process requires considerable skill and experience, besides the use of the best microtomes and knives. The Jung sliding microtome for wood sectioning is the best, but needs much heavier and more solid carriers. The carriers should have long antifriction-metal bearings and some device similar to that provided with the celloidin microtome for adjusting the object carrier to the desired plane of section. The success of working the microtome depends upon keeping the knife absolutely free from nicks and extremely sharp, in having all the bearings free from dust and dirt and well oiled, in regulating the angle at which the knife cuts, and in orienting the surface of the block in accordance with the hardness and structure of the wood.

5. USE OF CELLOIDIN.—The celloidin method is unnecessary in cutting thin sections of woody tissues except in the case of small fragments. Even the latter may often be cut successfully without imbedding by gluing to the carrier block with a few coats of 4 per cent. celloidin. In working with extremely soft woods, decayed woods, or in making careful studies of soft structures like the resin canals of the white pines, the celloidin method is useful.

6. CUTTING WITHOUT DEMINERALIZATION.—Sections of soft woods as thin as 5 μ may be cut after boiling without demineralization, but for this purpose an old knife which can be repeatedly honed and sharpened is needed.

7. STAINING.—For most purposes the hematoxylin-safranin double stain is the most serviceable; but in studying special structures, such as spiral markings and the pitting of conifers, unstained sections mounted in glycerin jelly are preferable. In fact, in any given problem experiment with the various steps of the method is necessary to secure the best results. When the structures are obscured by deposits of resinous materials, tannins, etc., these must often be removed to reveal the pitting and sculpture of the cell walls.—IRVING W. BAILEY, *Harvard University*.

VIVIPARY IN *TILLANDSIA TENUIFOLIA* L.

(WITH ONE FIGURE)

SMALL, in his *Flora S. E. United States*, gives the range of this species of Tillandsia as in swamps and about rivers in Georgia and Florida, also in tropic America. Its normal method of reproduction is by small, light, pointed seeds, to which are attached a bunch of hairs that spread out at the top horizontally, thus acting as a parachute for wind carriage. This in connection with the epiphytic life is the most suitable method by which the seeds can find lodgment upon some limb or tree trunk and grow attached some distance above the ground.

FIG. 1

Another method of reproduction by the adoption of a viviparous habit was discovered in this plant in a specimen brought from the south on the branch of a forest tree which was hung in the greenhouses of the University of Pennsylvania. In the three or four plants examined, it was found that the seeds in all cases (*fig. 1, b*) had germinated in the capsules (*fig. 1, a*), and that the seedling plants thus produced (*fig. 1, c*) had two or three short shoots, as well as four or five awl-shaped leaves arranged in the tufted manner of the mature plant. These small plants were ready to fall out of the cells of the capsule whenever the dry valves spread far enough apart to permit this discharge to take place. Whether this viviparous habit is shown by all the species of Tillandsia, I cannot state, but it seems to be a method by which additional surety is given to the perpetuation of the species. —JOHN W. HARSHBERGER, *University of Pennsylvania*.

CURRENT LITERATURE

BOOK REVIEWS

Studies in fossil botany

SCOTT's *Studies in fossil botany* has been the standard text in its field since 1900. It is not a text on paleobotany in the ordinary sense, but is restricted to the vascular plants, and in presenting them deals especially with their "morphological and evolutionary aspects." This means an expert culling of significant structures from the enormous mass of published paleobotanical material, and it has been of immense service to those morphologists who wish to use the historical background. During the present decade this kind of paleobotany has not only made notable advance in the precision of its methods, but it has made some very remarkable discoveries. The rapid progress in knowledge has compelled a second edition of SCOTT's *Studies*,[1] which has grown from a book of 533 pages to one of 683 pages, the illustrations having increased from 151 to 213.

To note even all of the important changes would be impossible in a brief review, and this is not necessary for those morphologists who have followed the literature of the subject since 1900; but the assembling of the results recorded in scattered papers is very impressive. A few of the advances that should be borne in mind even by the general morphologist may be noted.

The primary divisions of pteridophytes are increased in number by the admission of NATHORST's Pseudoborniales, discovered in the Upper Devonian of the arctic regions and described in 1902. These remarkable forms are known as yet only from impressions, but the stems are articulated, branching, probably creeping, bearing whorls of repeatedly dichotomous leaves with deeply pinnatifid leaflets and long loose strobili ("spikes") with whorled sporophylls resembling reduced foliage leaves.

The seedlike megasporangia of some of the paleozoic Lycopodiales, referred to briefly in the first edition, assume greater proportions in the second. The conclusion is becoming irresistible that no definition of a seed can be constructed which will exclude the megasporangiate structures of these ancient lycopods, and yet it is evident that they should not belong to the Spermatophyta. The definition of a great group can no longer be based upon a single character, but must be based upon a plexus of characters.

The presentation of paleozoic Filicales has been absolutely transformed. It is during the present decade that the "Pteridospermeae" have practically replaced the supposed paleozoic "ferns," and the present volume can claim only

[1] SCOTT, DUKINFIELD HENRY, Studies in fossil botany. Second edition. 8vo. pp. xxiv + 683. *figs. 213*. London: Adam and Charles Black. 1909. 10*s* 6*d*.

the Botryopterideae as sure representatives of paleozoic Filicales. It must always be remembered, however, that all the members of the great frond genera have not been proved to be seed plants, but they are all under suspicion.

Of course the "pteridosperms" are presented with a completeness that has not been possible heretofore. In traversing the evidence, SCOTT reaches the conclusion that every great frond genus is involved in the seed-bearing habit, and also in the discovery of stamens. It is of interest to note that the Crossotheca ("epaulet") type of stamens is probably the prevailing kind, and that the so-called Calymmatotheca ("cupule") type is likely to be the husk or cupule of seeds. The statement that *Gnetopsis elliptica*, which has been one of the guesses at a history for the Gnetales, is probably a seed of the Lyginodendreae relieves a very doubtful situation.

The perspective of the Cordaitales is increased by the recognition of Poroxylon and Pitys as intermediate in vascular structure between Lyginodendron and Cordaites, the gradation from the mesarch cylinder of the former to the endarch cylinder of the latter being very complete. As a consequence, the group Cordaitales is made to comprise Pityeae, Poroxlyeae, and Cordaiteae.

The mesozoic Bennettitales represent the second great vascular group that has been brought to light during the last decade, and their presentation is another new feature of the volume. WIELAND'S laborious researches have developed a knowledge of this extinct and widely distributed cycadean group that almost rivals that of the living groups. SCOTT inclines to follow WIELAND'S suggestion that the remarkable strobilus of Bennettitales must be taken into consideration in searching for the origin of the angiosperms, a view that is certainly not contradicted at present by the history of the two groups.

Perhaps the most significant suggestion in the volume is a proposed new classification of vascular plants. SCOTT evidently feels that the old division into pteridophytes and spermatophytes has about outlived its usefulness, a feeling doubtless shared by all morphologists who deal specially with the two groups. Whether the exact form of the suggestion will be acceptable or not remains to be seen, but the author is to be congratulated upon being bold enough to break the silence again. His suggestion is that vascular plants be grouped in three primary assemblages: *Sphenopsida* (Equisetales, Pseudoborniales, Sphenophyllales, Psilotales), *Lycopsida* (Lycopodiales), and *Pteropsida* (Filicales, Pteridospermeae, Gymnospermeae, and Angiospermeae). This crosses the old division, and the author expresses the opinion that "the traditional classification will no doubt continue to be used on grounds of convenience;" in fact, the two volumes, in which the present work also appears, bear the subtitles "Pteridophyta" and "Spermophyta." Pteropsida and Lycopsida are names proposed by JEFFREY, who made a similar proposition years ago. The former name is used as JEFFREY defined it; but the latter is limited to the Lycopodiales, as the only "typically microphyllous phylum." SCOTT does not think that the "sporangiophoric pteridophytes" (BOWER'S name for SCOTT'S Sphenopsida) show an affinity for the lycopods that would justify their inclusion under a common name.—J. M. C.

The mutation theory

In 1901 the first volume of DEVRIES'S *Mutationstheorie* was published, and was reviewed in this journal.[2] It put the doctrine of evolution upon an experimental basis and has been the stimulus of a large amount of important investigation. Such a contribution to biology is of the first order, whether the theory of mutation proves to be a general explanation of the origin of species or not. It has been unfortunate that many who are interested in evolution, even investigators, have not understood fully the theory of mutation, because they know of it only from condensed statements or reviews. This applies to all who do not read German or who do not read it with facility. This condition has now been remedied by the appearance of an Engish translation[3] of this great work. It is possible now for all English-reading biologists to judge of the theory from the full presentation by its author, and to substitute knowledge for superficial familiarity.

The full review of the original volume by this journal makes it necessary only to announce the appearance of the translation. And yet, eight years have elapsed since the theory was formally proposed and much could be said of its standing and influence. During the same period DEVRIES has twice visited the United States, and by conversation and lecture and personality has impressed himself and his views in a peculiar way upon American biologists. His influence upon scientific plant and animal breeding in this country has been very great, and the general result has been an increasing conviction that his views and his example have advanced biological science immensely.

The thesis of this first volume, as its author states, is the promulgation of the principle of unit characters. At the time of its announcement in 1900 this view was in opposition to the current belief; but the author is convinced that it has now gained almost universal acceptance. This conception that "the attributes of organisms consist of distinct, separate, and independent units" was derived chiefly from three sources: (1) an analysis of the processes of selection in practical plant breeding; (2) the experimental evidence afforded by Oenothera; and (3) Mendelism. This means that the origin of species by mutation is only one feature connected with the more fundamental conception of unit characters, and that a wide range of problems is opened up to experimental investigation. The only important change introduced into the translation is the incorporation of NILSSON'S results in the selection and improvement of cereals in Sweden.

The selection of translators could not have been more happy and effective; and the publishers are to be congratulated, not only for the worthy appearance of this book, but also for their real contribution to the advancement of knowledge in assuming the responsibility of publication.

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[2] BOT. GAZETTE **33**:236–239. 1902.

[3] DEVRIES, HUGO, The mutation theory; experiments and observations on the origin of species in the vegetable kingdom. Translated by J. B. FARMER and A. D. DARBISHIRE. Vol. I. The origin of species by mutation. 8vo. pp. xv+582. *colored pls. 6. figs. 119.* Chicago: The Open Court Publishing Company. 1909. $4.00.

The English translation will consist of two volumes, the second treating of the origin of varieties and also of the general problems of the origin of new forms. The second volume is announced to appear in April.—J. M. C.

MINOR NOTICES

Knuth's handbook.—The third volume of the English translation of KNUTH'S *Handbuch der Blutenbiologie* has just appeared[4] and completes the work. The original volumes and the two preceding volumes of the translation were reviewed in this journal,[5] so that the general scope and character of the work have been noted. The first volume is a general one, treating of the structure of flowers and of insects in relation to pollination. The second volume is a record of observations on flower pollination made in Europe and the arctic regions, arranged by natural orders and extending from "Ranunculaceae to Stylideae." The present volume continues the subject of the second and includes the natural orders from "Goodenovieae to Cycadeae." The encyclopedic character of the work may be judged by the fact that the second and third volumes contain observations on 1048 genera and 3112 species of plants. There is appended a systematic list of insect visitors recorded in these two volumes, arranged alphabetically and with the names of the plants visited, and the number of species reaches 2888.

Such a work cannot be reviewed, it can only be announced. The burden of translation must have been enormous, but it has introduced English-reading botanists to an immense body of facts, and may serve to stimulate work in this attractive field. The total cost of the three volumes ($24.25 in cloth, and $27.25 in half morocco) may seem somewhat prohibitive, but the work may be made available in libraries.—J. M. C.

A new laboratory guide.—The widespread interest in the teaching of botany in secondary schools is shown by the abundance of new textbooks and laboratory manuals. One of the latest of the latter is by CLUTE.[6] It is divided into two parts; the first deals with the usual topics in connection with the structure and activities of angiosperms, and the latter with the great plant groups. Its most striking features seem to be the small number of exercises, each of which is very comprehensive; the large number of suggestive questions; and the useful set of definitions accompanying each exercise. The directions to the pupil are mainly contained in the questions, and these are very numerous (99 in one of the exercises). Complete answering of these would demand careful study of the material used,

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[4] KNUTH, PAUL, Handbook of flower pollination, based upon HERMANN MÜLLER'S work "The fertilization of flowers by insects." Translated by J. R. AINSWORTH DAVIS. Vol. III. 8vo. pp. iv+644. *figs. 208*. Oxford: Clarendon Press. 1909. Cloth $8.75.

[5] BOT. GAZETTE **28**:280. 1899; **28**:432. 1899; **42**:494. 1906; **46**:63. 1908.

[6] CLUTE, WILLARD N., Laboratory botany for the high school. pp. xiv+177. Boston: Ginn & Co. 1909. 75 cents.

though possibly without sufficiently encouraging initiative. The book should be useful in classes somewhat above the grade of the first year in the high school, especially in those laboratories where very full directions are needed. At the same time, it is a question whether the author does not err, in common with many other writers of textbooks of this class, in giving structure complete predominance. In the study of living things it would appear that vital processes and relations should furnish the motive of the course. At any rate, it is scarcely fair to the pupil's perspective to relegate physiology to a few pages, in the nature of an appendix, with occasional references from the body of the text. One would prefer it to be an integral and important part of the laboratory directions.—W. L. EIKENBERRY.

North American flora.[7]—Volume XVI, part I, of this work contains an elaboration of the Ophioglossales-Filicales as follows: Ophioglossaceae by L. M. UNDERWOOD and R. C. BENEDICT; Marattiaceae by L. M. UNDERWOOD; Osmundaceae and Ceratopteridaceae by R. C. BENEDICT; Schizaeaceae, Gleicheniaceae, and Cyatheaceae (pars) by W. R. MAXON. New species are described in the following genera: Ophioglossum (1), Danaea (1), Marattia (1), Aneimia (7), Dicranopteris (1), and Cyathea (4).—J. M. GREENMAN.

NOTES FOR STUDENTS

Chromosome reduction in Oenothera.—DAVIS[8] publishes an account of chromosome reduction in *Oenothera grandiflora* which is a strong confirmation of the earlier work of GEERTS[9] on *O. Lamarckiana*, and of the reviewer on *O. Lamarckiana* and a number of its mutants and hybrids, especially *O. rubrinervis*.[10] As regards the main facts of reduction the results are the same in all three cases, and even many of the finer details of the reviewer's results are here corroborated, though there are evidently certain differences of behavior between *O. Lamarckiana*

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[7] North American Flora, Vol. XVI, part I, pp. 1–88. New York Botanical Garden. 1909.

[8] DAVIS, B. M., Cytological studies on Oenothera. I. Pollen development of *Oenothera grandiflora*. Annals of Botany **23**:551–571. *pls. 41, 42.* 1909.

[9] GEERTS, J. M., Beiträge zur Kenntnis der cytologischen Entwicklung von *Oenothera Lamarckiana*. Ber. Deutsch. Bot. Gesells. **26a**:608–614. 1908.

———, Beiträge zur Kenntnis der Cytologie und der partiellen Sterilität von *Oenothera Lamarckiana*. Rec. Travaux Bot. Néerlandais **5**:93–208. *pls. 5–22.* 1909.

[10] GATES, R. R., Pollen development in hybrids of *Oenothera lata* × *O. Lamarckiana*, and its relation to mutation. BOT. GAZETTE **43**:81–115. *pls. 2–4.* 1907.

———, Hybridization and germ cells of *Oenothera mutants*. BOT. GAZETTE **44**:1–21. *figs. 3.* 1907.

———, A study of reduction in *Oenothera rubrinervis*. BOT. GAZETTE **46**:1–34. *pls. 1–3.* 1908.

———, The behavior of chromosomes in *Oenothera lata* × *O. gigas*. BOT. GAZETTE **48**:179–199. *pls. 12–14.* 1909.

and *O. grandiflora*, as well as certain differences in interpretation, which will be pointed out.

The present account substantiates the earlier one in two fundamental points: (1) in synapsis the nuclear reticulum contracts into a ball, during which a more or less continuous thread is formed, but there is no evidence of a pairing of separate threads; (2) the thick spirem finally segments in such a way as to show that the chromosomes must have been arranged in a single series end-to-end. These are the two essentials of the method of heterotypic chromosome formation according to the account of FARMER and MOORE, MOTTIER, and others.

The author of the paper under review was formerly a strong advocate of the method of reduction by a lateral pairing of spirems previous to synapsis. This fact, together with the fact that he is the third investigator who has now given the same account as regards the essential points of chromosome reduction in Oenothera, places practically beyond question the presence of a telosynapsis (end-to-end pairing) instead of a parasynapsis (side-by-side pairing) in this genus.

In the nature of things, a lateral pairing of delicate threads previous to synapsis is more difficult to demonstrate than the cross-segmentation of a thick spirem after synapsis (see GATES **'08,** *figs. 22–26*). But ROSENBERG has reiterated his belief in a parasynapsis in Drosera, and has recently[11] brought forward some particularly clear evidence of a lateral pairing in that genus. A number of other investigators have given more or less convincing evidence of a parasynapsis in other forms. In the light of these facts it seems pretty evident that both these general methods of reduction occur in plants, a view which I have advanced in former papers. When cytologists become ready to admit that the evident differences in accounts of reduction are not all differences of interpretation, but are in many cases differences in fact, we shall have taken a forward step in the science and shall be ready to attack the problem of the meaning of the differences in chromatin behavior involved. To admit that *all* differences in accounts are due to real differences in chromatin behavior, would introduce laxity into interpretations which must necessarily always be critical, but nevertheless it must be admitted that in some of the more recent papers, clear and important differences in the method of the chromatin behavior have been established.

Several differences between DAVIS's account of reduction in *O. grandiflora* and my account of that process in the *O. Lamarckiana* forms may now be pointed out. They are partly differences of interpretation and partly differences of fact. DAVIS states that the chromatic bodies in the resting nuclei are probably prochromosomes, but this is evidently the statement of a pious wish rather than an observation of fact, for he himself finds variation in the size and number of such bodies. He has unfortunately failed to distinguish between the true synapsis or synizesis, which is familiar to all cytologists as the stage in which the thin and delicate spirem is contracted into a ball, and the second contraction phase,

[11] ROSENBERG, O., Cytologische und morphologische studien über *Drosera longifolia rotundifolia*. Kungl. Svenska Vetenskapsakad. Handl. **43**:1–64. *pls. 1–4*. 1909.

which comes much later in the history of reduction, after the spirem has undergone an enormous amount of shortening and thickening. In Oenothera it is a well-marked stage, immediately preceding the transverse segmentation of the spirem to form chromosomes; and separated from synizesis by a long interval, during which the spirem loosens its coils, enabling the rearrangement and thickening of the threads to go on.

The author's account of the heterotypic chromosomes in *O. grandiflora* shows an evident difference between this species and the *O. Lamarckiana* forms. In the former, rings are said always to be produced, although no adequate evidence of their method of origin is presented. While it is probable that many of the rings figured are really open at one end, or with the chromosomes merely in contact and not fused, yet a comparison of DAVIS's *figs. 27–30* with my *figs. 26–34* (*O. rubrinervis*, **'08**) shows that evidently the attraction which leads a chromosome to pair with its mate is greater in *O. grandiflora* than in *O. Lamarckiana* forms. This condition in *O. Lamarckiana* forms may have come about as a result of conditions of cultivation or hybridization, and, as I have pointed out elsewhere, may be directly connected with the mutation phenomena in *O. Lamarckiana*. Whether or not this is the case, I have shown that, as a result of this weak attraction between chromosomes, irregularities in chromosome distribution during reduction actually do occur.—R. R. GATES.

Cytology of the ascus.—BLACKMAN and Miss FRASER,[12] and more recently Miss FRASER[13] and Miss WELSFORD,[14,15] have concluded that in *Humaria granulata*, *Ascobolus furfuraceus*, and *Lachnea stercorea* a normal sexual process does not obtain. Instead of a fusion of sexual nuclei in the oogonium, a reduced sexual process occurs in which the female nuclei fuse in pairs with a subsequent asexual fusion in the ascus.

Still more recently FRASER and BROOKS[16] have studied further the process of nuclear division, methods of chromosome reduction, and spore formation in the above-mentioned pseudo-apogamous forms. They find in each of these plants two sorts of reducing divisions occurring in the ascus. The first two divisions are heterotypic and homeotypic respectively, and thus bring about a chromosome reduction in the manner described for higher plants. They regard this type of reduction as being associated with fertilization or its equivalent. The third

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[12] BLACKMAN, V. H., AND FRASER, HELEN C. I., On the sexuality and development of the ascocarp of *Humaria granulata*. Proc. Roy. Soc. London B **77**:354–368. 1906.

[13] FRASER, HELEN C. I., On the sexuality and development of the ascocarp of *Lachnea stercorea*. Annals of Botany **21**:349–360. 1907.

[14] WELSFORD, E. J., Fertilization in *Ascobolus furfuraceus*. New Phytol. **7**:156–161. 1907.

[15] FRASER, H. C. I., AND WELSFORD, E. J., Further contributions to the cytology of the Ascomycetes. Annals of Botany **22**:465–477. 1908.

[16] FRASER, H. C. I., AND BROOKS, W. E. ST. J., Further studies on the cytology of the ascus. Annals of Botany **23**:537–549. 1909.

division of the ascus nucleus, which differs little in appearance from the normal vegetative type of division, is described as brachymeiotic, in which half the heterotype number of chromosomes is present, thus bringing about, as is believed, a second reduction in the number of chromosomes. In *Ascobolus furfuraceus* and *Humaria granulata* they find that the eight chromosomes are paired throughout the second and also in the prophases of the third division. In *Lachnea stercorea*, in which the meiotic prophases were studied more in detail, the heterotype number of chromosomes is four, two chromosomes being much longer than the other two. The first numerical reduction occurs by an end-to-end union of the chromosomes, which are subsequently separated transversely. In the third metaphase four chromosomes are present. Two entire chromosomes, one long and one short, pass to each daughter nucleus.

Brachymeiosis is believed to differ markedly from meiosis in several particulars. In meiosis it is held that the most essential feature seems to be the union of two chromosomes to form so-called gemini, while brachymeiosis may be accomplished without visible union of the chromosomes, as described for *Lachnea stercorea*, and when pairing does occur in this division, as described for *Ascobolus furfuraceus* and *Humaria granulata*, the opportunity for interchange of material is held to be much less than in meiosis. It is believed, therefore, that it is possible to distinguish between sexual and asexual fusions by a study of the subsequent reduction divisions. (The reviewer cannot agree with these authors that the distinguishing characteristic of meiosis, at least the visible distinction, rests on the close association of the chromosomes. The conspicuous fact is the formation of tetrads or so-called four-parted chromosomes. The formation of tetrads does not necessarily imply any very close association of the constituent parts of two-parted chromosomes.)

In the opinion of the authors the parental allelomorphs and also those brought together by the nuclear fusion in the ascus may show varying degrees of association, and this is brought out by a text figure, in which the forms here studied are compared with several other Ascomycetes. In *Humaria granulata* pairing of the chromosomes is present in the pre-meiotic divisions, while this pairing occurs in the meiotic prophases in *Ascobolus furfuraceus*, thus resembling *Otidea aurantia* and *Peziza vesiculosa* in this respect.

The authors also find that an important part is played by vacuoles in spore delimitation. They find in Ascobolus a differentiation into dense and vacuolated areas, but also that the center and astral rays are essential to spore delimitation. After the third division the cytoplasm becomes traversed by an irregular series of vacuoles or areas of cleavage, which separates the sporeplasms. It is held that a substance flows out from the center, capable of producing alterations in the cytoplasm, probably an enzyme. A definite membrane is put about the sporeplasm, and a similar one limits the neighboring periplasm, with a clear space between, marking the old lines of cleavage. Although these observations support FAULL's views, they also support the view that the astral rays play an important part in spore delimitation.—J. B. OVERTON.

A new color guide.—Several years ago I was appointed on a committee, with Dr. A. W. MURRILL and the late Dr. L. M. UNDERWOOD, by the American Mycological Society (now united with the Botanical Society of America) to prepare a color guide adequate to the needs of botanists and mycologists. After working on it for about two years I learned of a similar work in preparation by Dr. ROBERT RIDGWAY, the well-known ornithologist and author of a nomenclature of colors (1886) which contains 186 colors, shades, and tints. After a consultation with Dr. RIDGWAY, and later with the other members of the committee, it was decided to leave the field of color work to him. I am now glad to announce that Dr. RIDGWAY has been particularly fortunate in securing competent publishers, who state that the work will probably be ready in about six months. The chemist of the firm is an expert in colors, and has in fact been engaged at odd times for several years in preparing for a similar work. Dr. RIDGWAY has been at the revision of his old work, as his time would permit, for about twenty years, and it is safe to say that no similar work has ever been prepared with the same degree of physical and mathematical precision. This will be better understood by those familiar with MICHELSON'S interferometer (an instrument by which it is possible to measure the wave-lengths of all light colors in millionths of a millimeter) when it is stated that each primary and secondary color in this work is a composite resulting from the measurement, in wave-lengths of light, of each color as represented in nine standard works upon the subject, the measurements being made by Professor P. G. NUTTING of the U. S. Bureau of Standards.

The work will contain 64 plates, each with 27 blocks of color, in three rows of nine blocks each, or a total of about 1350 blocks, the blocks being 1.25 by 2.5^{cm}, as in RIDGWAY'S first color work. These will be named so far as possible, it being manifestly impossible to name all of them satisfactorily; and in addition the horizontal rows will be numbered and the vertical rows lettered, intermediate figures and letters between each row being used to represent intermediate shades or tints when that degree of accuracy is necessary. Instead of the degree of variation between blocks of color in various parts of the work being very unequal, as in all previous color guides of any size, the intermediate shades, tints, and hues are determined by a uniform scale of percentages by the use of MAXWELL'S color wheel. There will be a probable edition of 2,500 copies, the volumes being about 14 by 20^{cm}, and 2.5^{cm} thick. For field use it is expected about 800 of these copies will have their margins trimmed and be bound in flexible leather binding, easily fitting a coat pocket. The price with board covers will be about $5.00. The only work extant which approaches this in any respect is the *Répertoire de couleurs* of the Société Française de Chrysanthémistes, containing 1385 color shades and tints, of which only about 365 are named, and no method is provided for designating the others. It consists of two bulky volumes of loose plates in portfolios; many of the intermediate shades, tints, and hues are too near alike; and a prohibitive import duty brings the price in this country to about $10.00. The paper is too heavy, and its form entirely unsuited for field work.—P. L. RICKER, *Bureau of Plant Industry, Washington, D. C.*

Monograph of the Elaeagnaceae.—This elaborate paper by SERVETTAZ extends through 420 pages, and is a remarkable attempt by a single student to investigate every phase of a whole family. Naturally it is encyclopedic, and therefore cannot be reviewed in any real sense, but its general contents may be indicated. The first part[17] is concerned exclusively with the taxonomy of the family. The author recognizes two tribes and three well-marked genera: Hippophaë, Shepherdia, and Elaeagnus. The first genus is represented by a single species and three subspecies, the second by three species, and the third by 39 species, numerous subspecies and varieties, several of which are new to science. The genus Elaeagnus is divided into two sections, based on the time of flowering and the persistency of the leaves, and a very clear key leads to the species under which is given further a key to the subspecies and occasionally to the varieties, thus greatly facilitating the use of the monograph, particularly in the interpretation of the subordinate categories. Bibliography and synonomy, for the most part, are very fully given, but the few citations of American exsiccatae suggest a paucity of material as well as unfamiliarity with the names of American collectors. For instance, on page 21 we find under *Shepherdia canadensis* "no. 1205 Waghome," which is doubtless intended for WAGHORNE. Again, on the same page is noted "Charlotte Vermont no. 2452 Hall et Harbour;" this citation needs verification; it suggests a record made from a misleading label (HALL AND HARBOUR collected this species in Colorado and distributed it under 506). Furthermore, under *S. argentea* we find "Oregon no. 2013 Cusik," which is meant for the collection of WILLIAM C. CUSICK. These are minor details; nevertheless, they are inaccuracies in citation, and such inaccuracies often lead to confusion and are sometimes difficult of elimination. The descriptions are well drawn, the keys are clear, and the text figures are numerous. On the whole, therefore, the taxonomic part of the work represents a comprehensive and commendable study of the group.—J. M. GREENMAN.

The second part[18] deals with "anatomy and biology." The three genera above named are treated separately under each head, so that any anatomical, morphological, or "biological" fact belonging to any one of them may be looked up as readily as in a card catalogue.

The chapter titles of this second part are essentially as follows: (1) germination and the vegetation of the adult plant, (2) anatomy and development of the root, (3) anatomy and development of the stem, (4) anatomy and development of the leaf, (5) anatomy and development of the flower, fruit, and seed, (6) discussion of affinities, (7) summary and conclusions.

To the morphologist, as distinct from the anatomist, the items of interest recorded are the development of abundant sterile tissue capping the solitary

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[17] SERVETTAZ, CAMILLE, Monographie des Eléagnacées. I. Systematik. Beih. Bot. Centralbl. **25**: 1–128. *figs. 15.* 1909.

[18] SERVETTAZ, Monographie des Eléagnacées. II. Anatomie et Biologie. *Ibid.* **25**: 129–420. *figs. 140.* 1909.

megaspore mother cell; the appearance of an ordinary tetrad; the formation of a chalazal haustorium by the embryo sac, into which the three persistent and very active antipodal cells pass; and the development of a massive supsensor from the filamentous proembryo. The anatomical details are fully presented, and are directed chiefly toward taxonomic distinctions. The "biological" topics deal with the influence of various conditions (especially mesophytic), the crystals of calcium oxalate, starch, tannins, and elaeagnin. The taxonomist will discover that the three genera are quite justifiable, and will receive no shock upon finding that the family belongs just where it is now located in the ENGLER sequence.—J. M. C.

Cretaceous conifers.—The remarkable results obtained by HOLLICK and JEFFREY from a study of the plant remains preserved in Cretaceous clays of Staten Island have now been published.[19] Much of the material consisted of fragments of lignite, and this required the development of a special technic, which is described. Structural material of leafy twigs and cone scales was also secured. The recent and rapid organization of vascular anatomy as a scientific instrument is well exemplified in this memoir, not only in the identification of material, but still more in the interpretation of its significance.

The descriptions of specimens include three genera of Abietineae, Pinus (3 spp., 2 new), Prepinus (1 sp.), and Pityoxylon (1 sp.); but most important is the surprising display of Araucarineae, 16 genera being included, 9 of which are described as new (*Androvettia*, *Raritania*, *Eugeinitzia*, *Pseudogeinitzia*, *Anomaspis*, *Sphenaspis*, *Dactyolepis*, *Pityoidolepis*, *Brachyoxylon*), the remaining 7 genera being Widdringtonites, Thuites, Brachyphyllum, Geinitzia, Protodammara, Araucariopitys, and Araucarioxylon.

The great interest of these results lies in their bearing on the relationship of araucarians to the other conifers. The remains investigated include many that have been referred heretofore, from the appearance of their leafy branches or of their cones, to Taxodineae, Cupressineae, and Podocarpineae. The conifers referred to these tribes are now shown, by the microscopic structure of their leafy twigs and cone scales, to be araucarians, and represent a special subtribe, for which the name Brachyphylloideae is proposed. They are characterized by a special type of wood, the Brachyoxylon type, which is said to "ally them with both the Araucarineae (in the narrower sense) and the Abietineae." The authors believe that the araucarians of today have come from abietineous ancestors through Brachyphylloideae. The species of Pinus investigated are in general more archaic than any living species, and Prepinus shows structural features more primitive than in any other living or extinct conifer.—J. M. C.

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[19] HOLLICK, ARTHUR, AND JEFFREY, EDWARD CHARLES, Studies of Cretaceous coniferous remains from Kreischerville, New York. Mem. N. Y. Bot. Garden **3**: viii + 138. *pls. 29.* 1909.

Enzymes of germination.—DELEANO[20] has made a somewhat disconnected study of the transformation of oil and the concomitant occurrence of various enzymes in the castor bean during the process of germination. The tables relating to the oil content of the seeds (freed from coats) on successive days of germination show that the oil content is practically constant during the first eight days of germination, after that decreasing so rapidly that by the fifteenth day it has nearly all disappeared. Samples equivalent to those used for analysis were taken each day, ground up, and subjected to autolysis in 50–100cc of water, with a little toluene, for ten hours. The oil was saponified with increasing rapidity as germination advanced. Thus on the first day no autolysis took place; on the fourth day 5 per cent.; and on the sixth day 98 per cent. of the oil was hydrolyzed in ten hours. The accumulation of fatty acids did not take place. From these experiments the author concludes, in agreement as he supposes with LECLERC DU SABLON, that the oil is not saponified in the cells, but that saponification takes place only when the correlation of the cells is destroyed. This conclusion is scarcely warranted, since the products of hydrolysis of the oil are probably removed as soon as formed and still further changed. This is the almost universal course of enzymic activity in living plants, while it is also universally true that only when the correlation of the cellular processes is interrupted can the activity of the enzymes be clearly demonstrated by the accumulation of the products of enzymic activity.

The relative abundance of some of the enzymes at successive stages of germination was also determined. Catalase increases at first and then decreases with the disappearance of the oil. The oxidases increase for a time and then remain fairly constant. The author also believes that he has shown the presence of a reducing enzyme. The work on these enzymes is not of sufficient extent to allow any general conclusions regarding their functions.—H. HASSELBRING.

Ambrosia fungi.—NEGER has been giving attention to the fungi associated with certain insects, which utilize them for food. The monilia-like cells that the insects eat he proposes to call ambrosia, a generic term like nectar, bee-bread, etc., and the fungi are to be designated as ambrosia fungi. In his first paper[21] he considers the ambrosia galls (a happy substitute for zoomycocecidia) produced by gall-mites of the genus Asphondylia, in which the insect undergoes its development from egg to imago. The gall cavity is lined with a hymenium-like layer of fungus filaments producing spherical monilia-like cells, the ambrosia. Later pycnidia are formed on the external surface of the gall after the insect has

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[20] DELEANO, N. J., Recherches chimiques sur la germination. Centralbl. Bakt. Parasit. Infectionskrank. **24**[2]:130–146. 1909.

[21] NEGER, F. W., Ambrosiapilze. Ber Deutsch. Bot. Gesells. **26a**:735–754. *pl. 12. figs. 2.* 1908.

escaped. The fungi peculiar to these galls belong to the genus Macrophoma, and are not referable to the species of Phoma that inhabit the same hosts.

In a second paper[22] NEGER treats the fungi associated with certain wood-boring beetles, *Xyloterus dispar*, *X. lineatus*, and *Hylecoetus dermestoides*, which form ambrosia upon the walls of their tunnels. The fungus related to Hylecoetus is probably a species of Endomyces; the other two are closely similar, but not identical, and are not identifiable. The species of Ceratostomella, which NEGER formerly mistook for ambrosia fungi in this case, are merely weeds in the fungus garden (as are also yeasts and bacteria), which have no part in producing the edible cells The larvae of these beetles have thus, in their mouths almost, nutr tious food abstracted by the vegetative mycelium from the more distant wood cells, instead of the relatively poor food, the wood itself; moreover, the borings are confined to the sap-wood, where the fungi find appropriate conditions for growth.—C. R. B.

Ovule and ovulate flower of Juglans.—BENSON and WELSFORD[23] have investigated the ovule and ovulate flower of Juglans in reference to the discordant results obtained by VAN TIEGHEM (1869) and NICOLOFF (1905). The "allied genera" examined for comparative study were Myrica, Carpinus, Morus, Urtica, and Rheum. In brief, it may be said that the account of VAN TIEGHEM was confirmed in all particulars. Interesting phases of "reduction" exhibited by the flowers of *Juglans regia* are as follows: (1) the origin of a dimerous condition from a trimerous, (2) barren placentae with a vascular supply, (3) one mode of the phylogenetic origin of the orthotropous basal ovule from an anatropous parietal type.

More extended conclusions deal with the so-called epigyny of the group considered, and with the ovule in angiosperms. The investigators find in the group "no trace of that form of epigyny which is brought about by the concavity of the axis and sinking and inclusion of the ovary within it," which description hardly applies to epigyny anywhere. It is concluded that the described epigyny of Amentiferae need not be regarded as an advanced character, and that the term had better be avoided. The conclusions as to the ovule of angiosperms are: (1) it is appendicular, (2) it is phylogenetically provided with a dual integument, and (3) the vascular supply may be compared with that of the outer integument or "cupule" of Lagenostoma.—J. M. C.

Aridity and evolution.—Of the external factors which have influenced or caused the evolution of the plant kingdom, MACDOUGAL[24] places much stress

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[22] NEGER, F. W., Ambrosiapilze II. Ber. Deutsch. Bot. Gesells. **27**:372–389. *pl. 17. figs. 2.* 1909.

[23] BENSON, M., AND WELSFORD, E. J., The morphology of the ovule and female flower of *Juglans regia* and of a few allied genera. Annals of Botany **23**:623–633. *figs. 8.* 1909.

[24] MACDOUGAL, D. T., Influence of aridity upon the evolutionary development of plants. Plant World **12**:217–231. 1909.

upon the abundance of the water supply. Assuming that free water was a very important agent in the origination of living matter, the development of the gametophytic stage of plant life is shown to be dependent upon the same factor. As aridity was encountered over extended land areas, the stress thus produced led to the development of the sporophyte with its vascular system and finally its seed habit. The earlier geological strata from arid areas contain no plant fossils, because at that period vegetation was characterized by the predominance of plants with separate gametophytes. Cycads and Bennettitales were the first plants to show xerophytic structures, and from their beginning the history of desert forms may be traced. Aridity, causing the reduction of surface area and the thickening of tissues, has evolved spinose and switchlike forms; while in the regions of most extreme aridity this evolutionary development has gone much farther, with the development of remarkable storage structures in leaf and stem organs. These succulent xerophytes represent the highest degree of differentiation in seed plants, and are found most abundantly in the very arid regions of the Karoo in South Africa, in the deserts of Brazil and Chili, in the arid regions of southern Mexico, and in the Sonoran and Chihuahuan deserts.—GEO. D. FULLER.

Algae in the laboratory.—Teachers will be interested in the practical methods which have made NIEUWLAND[25] so successful in growing algae in the laboratory. Large aquaria are not necessary, the best results often being obtained with gallon or two-gallon jars. The commonest mistake is to change the water, even the single change usually involved in bringing algae from the field to the laboratory often being disastrous. It is safer to bring the water in which the plants are growing. When necessary to renew the water, not more than one-fifth the volume should be added, and tap water should be allowed to run for a moment to avoid the introduction of undesirable salts. Bacterial decomposition is best prevented by introducing only a small amount of material, not more than one cubic inch to the gallon. As the material grows, the increasing amount can be accommodated because of the developing adaptation to the environment. A few plants of Chara are often desirable, since they prevent the water from becoming too hard, and a little Utricularia will rid the culture of undesirable animal life. When the algae disappear, the culture should not be thrown out, for the forms usually reappear after a time. Mud and sticks from spots where algae have been noticed may be brought into the laboratory in midwinter and soon yield vigorous cultures. Detailed directions are given for securing and keeping many of the most desirable laboratory types.—CHARLES J. CHAMBERLAIN.

A new maize from China.—The Department of Agriculture has been investigating a new type of corn received from near Shanghai, China, in March 1906,

[25] NIEUWLAND, J. A., Hints on collecting and growing algae for class work. Midland Naturalist 1:85–97. 1909.

and the results have just been published by COLLINS.[26] It is distinct from all hitherto known types in its erect and one-ranked leaf blades, in the development of its "silks" while still within the leaf sheath, and in its peculiar endosperm. The first two of these unique characters insure pollination and prevent the drying-out of the silks, the pollen being blown against the erect leaf blades and accumulating at their bases, and the silks pushing into these accumulations before they are exposed to the air. The endosperm is neither starchy nor horny, resembling the latter in location and hardness, but having the texture and optical properties of very hard wax, and hence the suggested name is "waxy endosperm." The xenia characters in hybrids showed that colored aleurone is dominant to transparent, yellow endosperm to white, and horny endosperm to waxy. This Chinese corn also raises the question as to whether maize was known in China before the discovery of America. That it is of American origin does not seem to be open to doubt, but Chinese literature of the sixteenth century refers to its introduction and widespread use, and this specialized type may have developed in China.—J. M. C.

Leaf structure of strand plants.—HARSHBERGER[27] has studied the leaves of twenty plants from the sandy shores of New Jersey and eleven from its salt marshes. All exhibit xerophytic structures and almost without exception have thickly cutinized epidermis. Four of the strand plants and six of the salt marsh species are succulents, while nine from the strand and three from the salt marsh have epidermal hairs. The drawings will form convenient plates of reference for students interested in these plants. It is to be regretted that they are not on a larger scale and that more attention has not been given to the differentiation of chlorophyll-bearing and mechanical tissues. The same criticism will apply to a previous paper by the same author[28] on the sand dune plants of Bermuda, which records observations for the leaves of seventeen plants. The author is confident that the various xerophytic leaf structures have been produced largely in response to intense illumination, exposure to strong winds, and in a few instances to the action of salt spray blown inland. He is inclined to overemphasize the influence of light, and says "chlorenchyma is converted into two kinds of tissues, palisade and spongy parenchyma, as a direct result of the unequal illumination of the leaf surfaces."—GEO. D. FULLER.

Ferments of fungi.—PRINGSHEIM and ZEMPTEN[29] have applied the Buchner method of extracting enzymes from yeast cells to the extraction of sugar-splitting

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[26] COLLINS, G. N., A new type of Indian corn from China. Bull. 161, Bureau Pl. Ind., Depart. Agric. pp. 30. *pls.* 2. 1909.

[27] HARSHBERGER, JOHN W., The comparative leaf structure of the strand plants of New Jersey. Proc. Amer. Phil. Soc. **48**: 72–89. *pls.* 2–5. 1909.

[28] ———, The comparative leaf structure of the sand dune plants of Bermuda. Proc. Amer. Phil. Soc. **47**: 97–110. *pls.* 1–3. 1908.

[29] PRINGSHEIM, H., AND ZEMPTEN, G., Studien über die Polysaccharide spaltenden Fermente in Pilzpresssäften. HOPPE-SEYLER'S Zeitschr. Physiol. Chemie **62**: 367–385. 1909.

ferments of various fungi. The results constitute a number of detached facts not readily summed up in a review; however, some of the main points may be noted. The press-extract of some of the fungi was found to be incapable of splitting any of the sugars used, while the residue was found capable of active fermentation, showing that the ferments in these cases are incapable of being separated from the rest of the cell by the Buchner process. In the case of *Aspergillus Wentii*, both the extract and the residue fermented cane sugar, milk sugar, maltose, cellobiose, and raffinose. It is of special interest to note that some of the fungi were able to utilize as food disaccharides, which neither the expressed juice nor the residue were able to ferment. These sugars were probably assimilated directly. The behavior of raffinose varied with different fungi. With *Aspergillus Wentii* this sugar was split into *d*-glucose, *d*-fructose, and *d*-galactose. In five other cases it was split into *d*-fructose and melibiose, and in three cases into *d*-galactose and cane sugar.—H. HASSELBRING.

The ring and cell wall of Oedogonium.—According to VAN WISSELINGH,[30] the cell wall of Oedogonium consists of two distinct layers; the outer containing little cellulose, but a large proportion of a characteristic membrane-forming material whose reactions are described, but whose chemical composition was not determined; while the inner wall is rich in cellulose and has a lamellate structure. The outer layer is lacking in the basal cell. The ring is a portion of the cell wall which arises by the intussusception of various membrane-forming materials, among which cellulose is prominent, especially in the centripetal portion. The rupture of the old wall and the stretching of the ring to form a new wall is about as usually described. The inner cellulose layer of the wall arises by apposition. The chemical tests and the observations are satisfactory so far as they go, but the subject is a much described and much discussed one, and it would seem that an investigator acquainted with technic, as VAN WISSELINGH is, could have advanced our knowledge much farther by adding a study of carefully stained sections.—CHARLES J. CHAMBERLAIN.

Anatomy of Saxegothaea.—TISON[31] has investigated the vascular anatomy of *Saxegothaea conspicua*, especially that of the ovulate strobilus. This genus has received much attention recently (NORÉN, STILES, THOMSON), and investigators have been impressed by its suggestion of araucarian affinities. TISON concludes, from the behavior and distribution of the two systems of bundles found in the megasporophyll (one serving the sporophyll and the other the ovule), that Saxeogothaea is more nearly related to the araucarians than to the podocarps, but that through Microcachrys it is so definitely connected with the latter that the araucarians, Saxegothaea, and the podocarps should constitute a single group,

[30] WISSELINGH, C. VAN, Ueber den Ring und die Zellwand bei Oedogonium. Beih. Bot. Centralbl. **23**: 157–190. *pls. 13–16.* 1908.

[31] TISON, A., Sur le *Saxegothaea* Lndl. Mém. Soc. Linn. Normandie **23**: 139–160. *pls. 9, 10.* 1909.

with the three subdivisions indicated. This conclusion depends upon the view that the structure of the ovulate cone, especially its vascular structure, is the paramount feature in determining relationship. An interesting incidental suggestion (following BERTRAND) is that the aril of Saxegothaea is the equivalent of the ligule of Araucaria and the ovuliferous scale of other conifers.—J. M. C.

A primitive lichen.—*Botrydina vulgaris*, regarded in general as a problematical green alga, has now been investigated by Miss ACTON.[32] It occurs as dark green, globular structures, which in the material examined were covering the shoots of a moss and a liverwort. Each one of these structures proved to consist of a central group of algal cells imbedded in mucilage, which in turn was traversed by investing fungal hyphae that formed also a colorless envelope of considerable thickness. The cultures showed that both the alga and the fungus are "able to develop quite well apart, and multiplication of Botrydina is probably due to this." Since this structure consists of an alga and a fungus growing symbiotically, the conclusion is that it should be regarded as a lichen, and that it is "possibly one of the most primitive of existing lichens." The alga and the fungus were both determined, and the habitat is said to be "in damp shady situations among various bryophytes, generally on rocks, but sometimes on damp ground."—J. M. C.

Movements of Myriophyllum leaves.—WÄCHTER has recorded[33] some interesting phenomena regarding the young leaves of *Myriophyllum proserpinacoides*. The leaves of both the land and water form of this plant have already been known to execute so-called sleep movements, so long as they are capable of growth; and such movements would be very properly called photonastic.[34] In studying these movements WÄCHTER has discovered that leaves which had almost or quite ceased to respond to light would resume these curvatures if the shoot were decapitated. This seems to be a phenomenon analogous to the reactivation of growth in the nodes of grasses under a gravity stimulus, and still more like the reaction of certain conifers to decapitation, though different in details from either. It has also relations to the excitation of growth by a wound stimulus, and compensative growth such as that in Streptocarpus when the big cotyledon is removed or incased in plaster and the small one resumes its development.—C. R. B.

The mycorhiza of Cordaites.—*Amyelon radicans* is a root of the Coal Measures, which has been shown to belong to Cordaites. It bears such remarkable and irregularly arranged bunches of lateral roots, that OSBORN[35] has examined

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[32] ACTON, ELIZABETH, *Botrydina vulgaris* Brebisson, a primitive lichen. Annals of Botany **23**:579–585. *pl. 44.* 1909.

[33] WÄCHTER, W., Beobachtungen über die Bewegungen der Blätter von *Myriophyllum proserpinacoides*. Jahrb. Wiss. Bot. **46**:418–442. *figs. 2.* 1909.

[34] Cf. BOT. GAZETTE **48**:313. 1909.

[35] OSBORN, T. G. B., The lateral roots of *Amyelon radicans* Will., and their mycorhiza. Annals of Botany **23**:603–611. *pls. 46, 47.* 1909.

them to discover if these bunches might correspond in any way with the "root tubercles" of recent plants. As only one such case has been recorded from the Coal Measures, the result is of special interest. These lateral roots are found to have a thick cortex divisible into two regions, the inner of which contains dark cells that show evident fungal hyphae. The fungus occurs in knots of non-septate hyphae that bear sometimes terminal vesicles, but there was no trace of any spore-formation. The conclusion is reached that "Cordaites was probably a tree inhabiting saline swamps, and having bunches of coralline rootlets on its roots, such as are known to occur in many recent plants growing under similar conditions."—J. M. C.

Anatomy of Equisetum.—EAMES[36] has discovered that although the xylem of Equisetum is centrifugal throughout the vegetative stem, it is also centripetal in the axial bundles of the strobilus and of the sporophylls; in the former the bundles are "weakly mesarch," in the latter "strongly so." This suggests that the most primitive representatives of Equisetales had well-developed centripetal wood, and connects them with such ancient forms as Sphenophyllales, already suggested by SCOTT's discovery of centripetal wood in a calamite. All the large groups of pteridophytes are now known to possess centripetal wood, so that "such bundles in higher plants can be of no other phylogenetic value than as indicating general cryptogamic affinities." At the same time, Equisetum confirms the value of the leaf gap as a phylogenetic character, since in no case does the passage of a leaf trace from the stele leave a gap.—J. M. C.

Protection against light.—MARLOTH describes some very remarkable ways in which a few African desert plants reduce the amount of light which the green tissues of their leaves receive.[37] He refers to three categories: (1) plants with fleshy and green leaves, having membranous stipules which extend beyond and conceal them; (2) plants with fleshy and green leaves, without stipules, but invested by the dried-up remnants of the older leaves; (3) plants with windowed leaves. This most curious arrangement is characteristic of plants with very fleshy leaves whose blunt, plane, or erose tips alone reach the surface of the soil, the body of the leaf being completely buried. This exposed tip lacks chlorophyll, and through this as through a window the light reaches the green tissue, which is restricted to the sides of the fat leaf. Several species of Mesembryanthemum have this peculiarity.—C. R. B.

"Transpiration" in aquatics.—Under a similar misleading title THODAY and SYKES[38] present a brief account of a few experiments that show movement of

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[36] EAMES, ARTHUR J., On the occurrence of centripetal xylem in Equisetum. Annals of Botany **23**: 587–601. *pl. 45.* 1909.

[37] MARLOTH, R., Die Schutzmittel der Pflanzen gegen übermässige Insolation. Ber. Deutsch. Bot. Gesells. **27**: 362–371. *figs. 2.* 1909.

[38] THODAY, D., AND SYKES, M. G., Preliminary observations on the transpiration current in submerged water-plants. Annals of Botany **23**: 635–637. 1909.

water through the stems of submerged aquatics. To call such a stream "the transpiration current" is manifestly absurd, unless one changes the meaning of the word transpiration. It will be remembered that others have found evidence of like movements, so that these new experiments only add somewhat clearer evidence as to its existence, which the most elementary consideration of the physical conditions would lead one to expect. Yet these authors naïvely say: "Probably external conditions also affect the results; this point we hope to investigate later." This really is the fundamental point: does not the heating of the leaves create the conditions for the circulation of water as truly in this case as in a house heating system?—C. R. B.

Fixation of free nitrogen.—POLLACCI reports in a preliminary note[39] that in a large number of experiments he has demonstrated the fixation of free nitrogen in such plants as lichen, salvinia, azolla, fern prothallia, and duckweed. The increase of total N in a few cases cited amounts to 33–67 per cent. The full paper will be awaited with interest. POLLACCI has a heavy weight of adverse evidence to counterbalance. He indicates that the contradictory results of the earlier observers, e. g., BOUSSINGAULT and VILLE, were probably due to differences in the capacity of different plants for this fixation. It is to be remembered, however, that all the recent evidence under improved chemical methods is adverse to the idea that ordinary plants are able to utilize N_2.—C. R. B.

Prothallium and embryo of Danaea.—CAMPBELL[40] has made a preliminary investigation of the prothallium and embryo in several species of Danaea secured in Jamaica. The archegonia are remarkable for the imperfect development of the ventral canal cell, which in many cases could not be demonstrated at all. The fertilized egg becomes elongated in the direction of the axis of the archegonium before the first division. The hypobasal cell does not divide or there is a single division, resulting in a short suspensor, all of the regions of the embryo arising from the epibasal cell. This cell gives rise to somewhat irregular quadrants, the two lower ones forming the foot, and the two upper giving rise to stem tip and leaf, and later to the root.—J. M. C.

Chromosomes of Hyacinthus.—Miss HYDE[41] finds that in Hyacinthus in the prophase of the heterotypic mitosis the spirem twists into 8 loops which become 8 chromosomes. The loops break apart at the center so as to form 8 bivalent chromosomes. When fully formed, the chromosomes show a striking difference in size, 4 being comparatively large, 3 small, and the remaining one intermediate.

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[39] POLLACCI, G., Ricerche sull' assimilazione dell' azoto atmosferico nei vegetali. Atti Ist. Bot. Univ. Pavia II. **13**:351–354. 1909.

[40] CAMPBELL, D. H., The prothallium and embryo of Danaea. Preliminary note. Annals of Botany **23**:691. 1909.

[41] HYDE, EDITH, The reduction division in the anthers of *Hyacinthus orientalis* Ohio Naturalist **9**:539–544. *pl. 32.* 1909.

Miss HYDE believes that the two chromosomes which must have united to form a bivalent chromosome are alike in size and shape, and that they represent paternal and maternal bodies. If extended observation should show that the differentiation of chromosomes shown in the figures is constant, this form would repay a thorough investigation.—CHARLES J. CHAMBERLAIN.

Apogamy in Oenothera.—In connection with his cultures of Oenothera, GATES[42] has discovered apogamy in *O. lata*, one of the mutants of *O. Lamarckiana*. The anthers of *O. lata* from the Amsterdam cultures are persistently sterile, and this fact, associated in certain other genera with apogamy, suggested the possibility of apogamy in this form. To determine this, the anthers and styles of several flowers (on one individual) were removed and the flowers bagged as usual in making guarded crosses. All of these flowers gave negative results except one, which produced three fairly good seeds. The cytological investigation necessary to substantiate and explain this result is being made.—J. M. C.

Heath vegetation.—Some of the ecological similarities of the coastal and barren regions of New York and New Jersey and the heath of Luneneburg are pointed out by LIVINGSTON,[43] who would account for the desert-like aspect of the vegetation of the heath of Luneneburg by the too rapid drainage of the soil and the short growing season. The areas of bog or marsh found scattered through the heath are also physiologically dry, perhaps mainly because of the toxic organic matter present in the soil; hence such areas differ little in aspect from the heath. Both the heath and the moor are dominated by *Calluna vulgaris*, while *Juniperus communis* is conspicuous on the open heath.—GEO. D. FULLER.

The "knee joint" of Mougeotia.—Observations upon several species of Mougeotia indicate to NIEUWLAND[44] that the prevalent interpretation of "knee joints" as a stage in conjugation is incorrect, for the joints are present only in vegetative stages and never in typically conjugating material. Usually the cells of the filament hold together so firmly that the cells break through the middle rather than separate at the ends, but in material with the knee joints, the cells are easily dissociated, and, succeeding the appearance of the joints, the amount of material increases enormously, so that the joints seem to be related to vegetative multiplication.—CHARLES J. CHAMBERLAIN.

Absorption of water by leaves.—In a lecture before the Royal Horticultural Society of London,[45] HENSLOW presented reasons derived from the older experiments and some recent ones by himself (which, by the way, are not all well con-

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[42] GATES, R. R., Apogamy in Oenothera. Science N. S. **30**: 691–694. 1909.

[43] LIVINGSTON, B. E., The heath of Luneneburg. Plant World **12**: 231–240. 1909.

[44] NIEUWLAND, J. A., The "knee joint" of species of Mougeotia. Midland Naturalist **1**: 82–84. 1909.

[45] HENSLOW, G., On the absorption of rain and dew by the green parts of plants. Jour. Roy. Hort. Soc. London **34**: 167–178. 1908.

ceived or convincing) that the leaves of plants admit water. He therefore justifies the practice of spraying the foliage. The most positive evidence that leaves admit water, as well as an indication of the magnitudes involved, is to be found in the cuticular evaporation. This does not seem to have occurred to HENSLOW, perhaps because he does not conceive of absorption and evaporation as a physical process.—C. R. B.

Imbedded antheridia in Dryopteris and Nephrodium.—In cultures of these two ferns to obtain apogamy, Miss BLACK[46] discovered "imbedded antheridia" in prothallia of both species, that is, such antheridia as occur in the eusporangiate forms. Although apogamy was not induced, these antheridia are of sufficient interest to justify the cultures. The association of the two kinds of antheridia in the same form, dependent upon conditions, is well known in Equisetum, but it does not seem to have been recorded before for ferns.—J. M. C.

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[46] BLACK, CAROLINE A., The development of the imbedded antheridium in *Dryopteris stipularis* (Willd.) Maxon and "*Nephrodium molle*." Bull. Torr. Bot. Club **36**: 557–571. *pls. 26–28.* 1909.